

RECOMBINANT DNA RESEARCH

Volume 17

Documents Relating to
"NIH Guidelines for Research
Involving Recombinant DNA Molecules"
December 1992–June 1993

January 1995



U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service National Institutes of Health

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Prepared by the
Office of Recombinant
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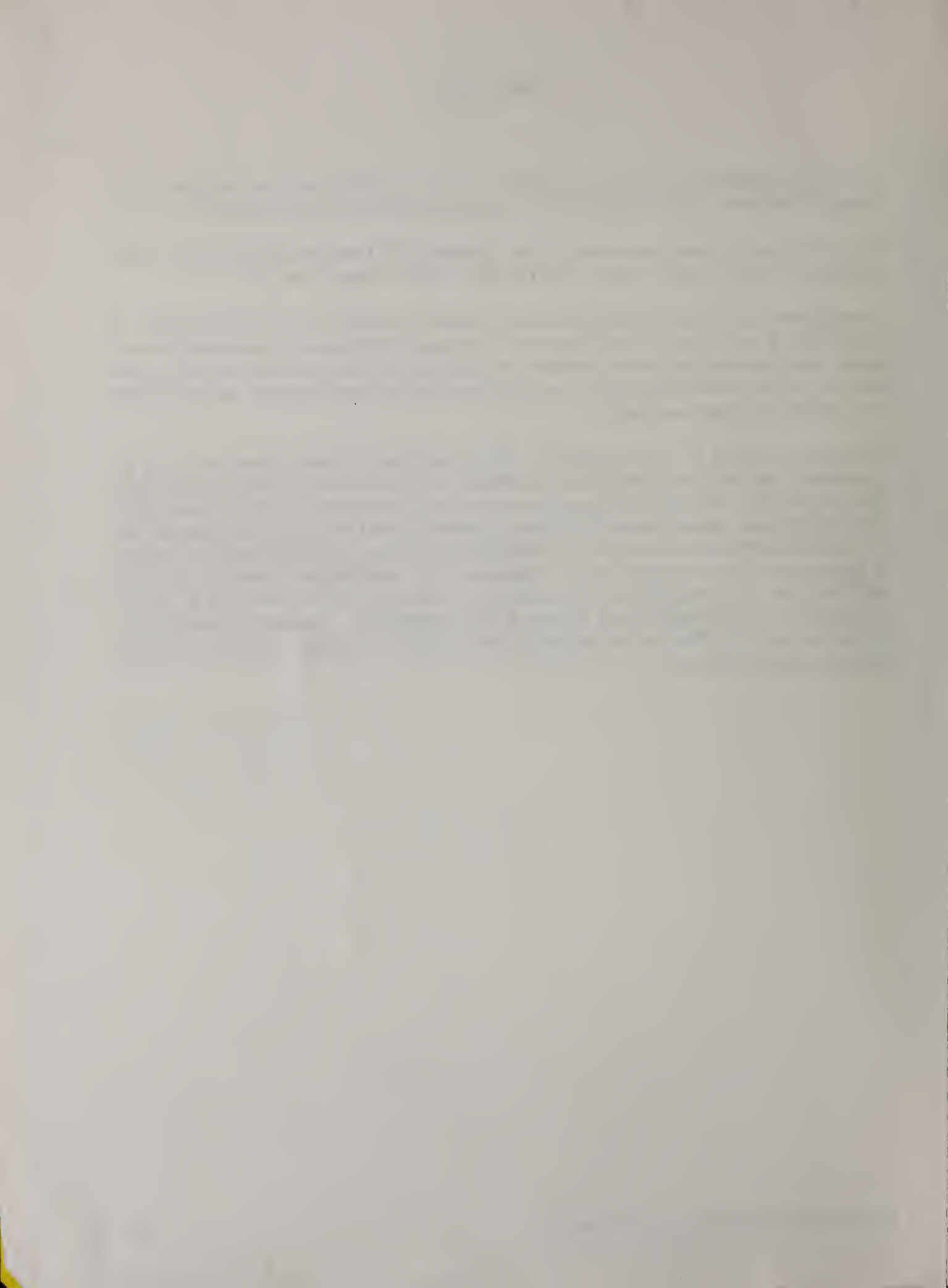
PREFACE

This is the seventeenth in a series constituting a "public record" of activities in regard to the National Institutes of Health (NIH) Guidelines for recombinant DNA research.

The first sixteen volumes cover events from February 1975 through December 1992. This seventeenth volume covers events from December 1992 through June 1993.

Starting from Volume 14, human gene transfer/therapy protocols that have been approved by the NIH Recombinant DNA Advisory Committee, are collected. Documents of each human study include the clinical protocol, the Informed Consent document describing the study in a nontechnical language, both scientific and nontechnical abstracts, and curriculum vitae of the principal investigators.

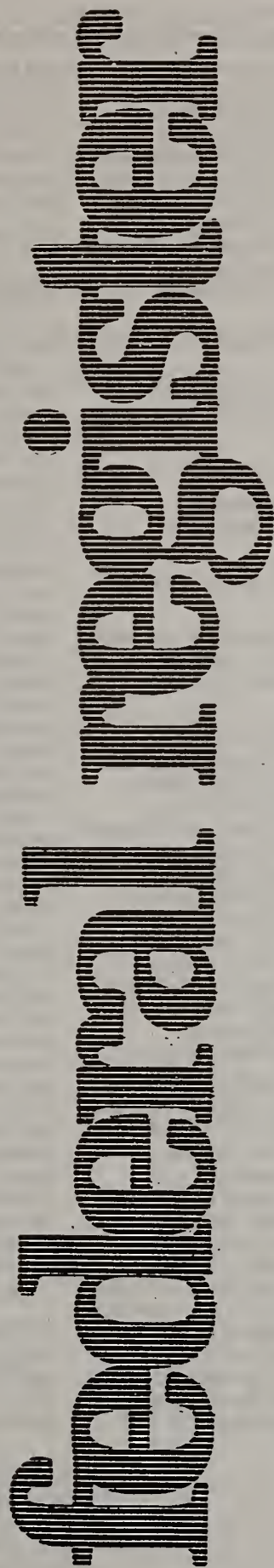
Volumes 1 through 5 in this series and the Environmental Impact Statement may be purchased from the Superintendent of Documents, U.S. Government Printing Office (GPO), Washington, DC 20402 or GPO bookstores in selected cities throughout the United States. They may also be viewed in some 600 public libraries of the GPO depository system. The GPO stock numbers are: Volume 1, 017-004-00398-6; Volume 2, 017-040-00422-2; Volume 2 Supplement (Environmental Impact Statement), 017-040-00413-3; Volume 3, 017-040-00429-0; Volume 3 Appendices, 017-040-0043-3; Volume 4, 017-040-00443-5; Volume 4 Appendices 017-040-00422-7; and Volume 5, 017-040-00470-2. Volumes 6 through 16 are available from the Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, MD 20892, USA.



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Wednesday
December 30, 1992

Part VIII

**Department of
Health and Human
Services**

National Institutes of Health

Recombinant DNA; Notices

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

Recombinant DNA Advisory Committee; Meeting

Pursuant to Public Law 92-463, notice is hereby given of a meeting of the Recombinant DNA Advisory Committee on January 14, 1993. The meeting will be held at the National Institutes of Health (NIH), Building 1, Wilson Hall, 9000 Rockville Pike, Bethesda, Maryland 20892, starting at approximately 9 a.m. on January 14, 1993, to adjournment at approximately 3 p.m. The meeting will be open to the public to discuss a proposed major action under the *NIH Guidelines for Research Involving Recombinant DNA Molecules* (51 FR 16958) involving an addition to Appendix D regarding a human gene therapy protocol and other matters to be considered by the Committee.

Attendance by the public will be limited to space available. Members of the public wishing to speak at this meeting may be given such opportunity at the discretion of the Chair.

Dr. Nelson A. Wivel, Director, Office of Recombinant DNA Activities, National Institutes of Health, Building 31, room 4B11, Bethesda, Maryland 20892, Phone (301) 496-9838, FAX (301) 496-9839, will provide materials to be discussed at this meeting, roster of committee members, and substantive program information. A summary of the meeting will be available at a later date.

OMB's "Mandatory Information Requirements for Federal Assistance Program Announcements" (45 FR 39592, June 11, 1980) requires a statement concerning the official government programs contained in the *Catalog of Federal Domestic Assistance*. Normally NIH lists in its announcements the number and title of affected individual programs for the guidance of the public. Because the guidance in this notice covers not only virtually every NIH program but also essentially every Federal research program in which DNA recombinant molecule techniques could be used, it has been determined not to be cost effective or in the public interest to attempt to list these programs. Such a list would likely require several additional pages. In addition, NIH could

not be certain that every Federal program would be included as many Federal agencies, as well as private organizations, both national and international, have elected to follow the *NIH Guidelines*. In lieu of the individual program listing, NIH invites readers to direct questions to the information address above about whether individual programs listed in the *Catalog of Federal Domestic Assistance* are affected.

Dated: December 24, 1992.

Susan K. Feldman,

Committee Management Officer, NIH.

[FR Doc. 92-31762 Filed 12-29-92; 8:45 am]

BILLING CODE 4140-01-M

Recombinant DNA Research: Proposed Actions Under the Guidelines

AGENCY: National Institutes of Health, PHS, DHHS.

ACTION: Notice of proposed action under the *NIH Guidelines for Research Involving Recombinant DNA Molecules* (51 FR 16958).

SUMMARY: This notice sets forth a proposed action to be taken under the *National Institutes of Health (NIH) Guidelines for Research Involving Recombinant DNA Molecules*. Interested parties are invited to submit comments concerning these proposals. This proposal will be considered by the Recombinant DNA Advisory Committee (RAC) at its meeting on January 14, 1993. After consideration of this proposal and comments by the RAC, the Director of the National Institutes of Health will issue decisions in accordance with the *NIH Guidelines*.

DATES: Comments received by January 8, 1993, will be reproduced and distributed to the RAC for consideration at its January 14, 1993, meeting.

ADDRESS: Written comments and recommendations should be submitted to Dr. Nelson A. Wivel, Director, Office of Recombinant DNA Activities, Building 31, room 4B11, National Institutes of Health, Bethesda, Maryland 20892, or sent by FAX to 301-496-9839.

All comments received in timely response to this notice will be considered and will be available for public inspection in the above office on weekdays between the hours of 8:30 a.m. and 5 p.m.

FOR FURTHER INFORMATION CONTACT:

Background documentation and additional information can be obtained from the office of Recombinant DNA Activities, Building 31, room 4B11, National Institutes of Health, Bethesda, Maryland 20892, (301) 496-9838.

SUPPLEMENTARY INFORMATION: The NIH will consider the following action under the *NIH Guidelines for Research Involving Recombinant DNA Molecules*:

In a letter dated December 7, 1992, Dr. Ivor Royston, San Diego Regional Cancer Center, San Diego, California, requested approval for a human gene therapy protocol. The title of this protocol is: *Phase I Study of Cytokine Therapy of Cancer, Active Immunotherapy of Glioblastoma with Tumor Cells or Fibroblasts Genetically Modified to Secrete Interleukin-2*.

OMB's "Mandatory Information Requirements for Federal Assistance Program Announcements" (45 FR 39592, June 11, 1980) requires a statement concerning the official government programs contained in the *Catalog of Federal Domestic Assistance*. Normally NIH lists in its announcements the number and title of affected individual programs for the guidance of the public. Because the guidance in this notice covers not only virtually every NIH program but also essentially every Federal research program in which DNA recombinant molecule techniques could be used, it has been determined not to be cost effective or in the public interest to attempt to list these programs. Such a list would likely require several additional pages. In addition, NIH could not be certain that every Federal program would be included as many Federal agencies, as well as private organizations, both national and international, have elected to follow the *NIH Guidelines*. In lieu of the individual program listing, NIH invites readers to direct questions to the information address above about whether individual programs listed in the *Catalog of Federal Domestic Assistance* are affected.

Dated: December 24, 1992.

Jay Moskowitz,

Associate Director for Science Policy and Legislation, NIH.

[FR Doc. 92-31763 Filed 12-29-92; 8:45 am]

BILLING CODE 4140-01-M

RECOMBINANT DNA ADVISORY COMMITTEE

Minutes of Meeting

January 14, 1993

**U.S. DEPARTMENT OF HEALTH
AND HUMAN SERVICES
Public Health Service
National Institutes of Health**

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DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

RECOMBINANT DNA ADVISORY COMMITTEE

MINUTES OF MEETING¹

January 14, 1993

The Recombinant DNA Advisory Committee (RAC) was convened for its fifty-second meeting at 9:00 a.m. on January 14, 1993, at the National Institutes of Health, Building 1, Wilson Hall, 9000 Rockville Pike, Bethesda, Maryland 20892. Dr. LeRoy B. Walters (Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public. The following were present for all or part of the meeting:

Committee members:

Nancy L. Buc, Weil, Gotshal, and Manges
Alexander Capron, University of Southern California (Conference Phone)
Ira H. Carmen, University of Illinois
Gary A. Chase, Johns Hopkins University
Patricia A. DeLeon, University of Delaware
E. Peter Geiduschek, University of California, San Diego (Conference Phone)
Robert Haselkorn, The University of Chicago (Conference Phone)
Susan S. Hirano, University of Wisconsin
Donald J. Krogstad, Tulane University School of Public Health
Brigid G. Leventhal, Johns Hopkins Hospital
Abbey S. Meyers, National Organization for Rare Disorders
A. Dusty Miller, Fred Hutchinson Cancer Research Center (Conference Phone)
Robertson Parkman, Childrens Hospital of Los Angeles
Leonard E. Post, Parke-Davis Pharmaceutical Division
Moselio Schaechter, Tufts University School of Medicine (Conference Phone)
LeRoy B. Walters, Kennedy Institute of Ethics, Georgetown University
Doris T. Zallen, VA Polytechnic Institute & State University

Executive secretary:

Nelson A. Wivel, National Institutes of Health

A committee roster is attached (Attachment).

¹The RAC is advisory to the National Institutes of Health (NIH), and its recommendations should not be considered as final or accepted. The Office of Recombinant DNA Activities should be consulted for NIH policy on specific issues.

Non-voting agency representatives:

Henry I. Miller, Food and Drug Administration
Kathy Hudson, Office of the Assistant Secretary for Health

Liaison Representative:

Daniel Jones, National Endowment for the Humanities

National Institutes of Health staff:

Bobbi Bennett, OD
Frederick Bonkovsky, CC
Norman Braveman, NIDR
Samuel Broder, NCI
Diane Bronzert, NCI
Sarah Carr, OD
Bruce Chabner, NCI
Sandy Chamblee, OD
Phillip Chen, OD
Mary Chunko, OD
John Diggs, OD
Tom Flavin, OD
MaryEllen Franko, NCI
William Gartland, NIDR
Mary Groesch, OD
Bernadine Healy, OD
Christine Ireland, OD
Susan Jenks, NCI
Kris Kiser, OD
Robert Lanman, OD
Becky Lawson, OD
Lance Liotta, OD
Jack Mahoney, OD
Richard Morgan, NHLBI
Jay Moskowitz, OD
Joan Porter, OD
Al Sandler, OD
Joanna Schneider, OD
Janet Smith, OD
Frances Taylor, NINDS
Debra Wilson, OD

Others:

Paul Aebersold, Food and Drug Administration
French Anderson, University of Southern California
James Barrett, Genetic Therapy, Inc.
Liz Bowie, The Baltimore Sun
Ann Driscoll, Fox, Bennett & Turner
Michelle Durand, The French Embassy
Ted Duvall, Medical News Network
Jeffrey Fox, Science News and Biotechnology
Diane Gershon, Nature
Paul Goldberg, The Cancer Letter
Kurt Gunter, Food and Drug Administration
Husband of Dr. Royston's Patient
Connie Lew, General Public
Richard Kozaic, Science Magazine
Alex Kuta, Food and Drug Administration
Charles Marwick, American Medical Association Journal
Katharine Matthews, Foundation on Economic Trends
Gerard McGarrity, Genetic Therapy, Inc.
Dori Meinert, Copley News Service
Robert Moen, Genetic Therapy, Inc.
Robert Murray, Howard University
Richard Norling, ABC Primetime Live
John Parker, The Blue Sheet
Raj Puri, Food and Drug Administration
Joyce Reilly, ABC Primetime Live
William Robinson, The Center for Applied Research
Ivor Royston, San Diego Cancer Research Center
Tomiko Shimada, Ambience Awareness International, Inc.
Robert Sobol, San Diego Cancer Research Center
Jennifer Sutton, Association of American Medical Colleges
Larry Thompson, Medical News Network
Paul Tolstoshev, Genetic Therapy, Inc.
Rick Vernard, Associated Press
Sue Walters, Kennedy Institute
Julie Wakefield, U.S. Medicine
R. Michael Williams, Cancer Treatment Centers
Janet Woodcock, Food and Drug Administration
William Yates, ABC Primetime Live
Kathryn Zoon, Food and Drug Administration

I. CALL TO ORDER

Dr. Wivel, Executive Secretary of the Recombinant DNA Advisory Committee (RAC), welcomed the new Chair, Dr. Leroy Walters, Director of the Center for Bioethics, Kennedy Institute of Ethics, Georgetown University, Washington, D.C. Dr. Wivel noted that Dr. Walters was a member of the working group that designed the *Points to Consider in the Design and Submission of Protocols for the Transfer of Recombinant DNA into the Genome of Human Subjects (Points to Consider)* and served as Chair of the Human Gene Therapy Subcommittee (HGTS) throughout its existence.

II. OPENING REMARKS--DR. WALTERS

Dr. Walters presented a brief historical background on recombinant DNA research and gene therapy. In 1973, Maxine Singer wrote a letter to *Science* entitled, *Guidelines for DNA Hybrid Molecules*. In 1974, Paul Berg and 10 fellow committee members from the National Research Council wrote a letter to *Science* entitled, *Potential Hazards of Recombinant DNA*. In 1975, there was a meeting in Asilomar to discuss the hazards associated with recombinant DNA; the RAC was established following the Asilomar meeting. In 1976, the first version of the *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)* was published. In 1982, the Presidents Commission for the Study of Ethical Problems in Medicine and Biomedical Behavioral Research published a report entitled, *Splicing Life*. In 1984, the RAC established the Working Group on Human Gene Therapy to discuss issues relevant to the review and approval of human gene therapy protocols. In 1985, the first version of the *Points to Consider* was published in the *Federal Register*. In 1988, the RAC and NIH Director approved the first human gene transfer protocol. In 1990, the RAC and NIH Director approved the first two human gene therapy protocols. In December 1992, the 34th through the 37th human gene transfer/therapy protocols were recommended for approval by the RAC to the NIH Director. Also in December 1992, the RAC confronted the issue of compassionate use of gene therapy for the first time.

Dr. Walters explained that the purpose of providing this historical review is to remind the RAC that revolutionary advances have been made possible by recombinant DNA techniques within the last 20 years. The RAC has a long tradition and distinguished history. The RAC has survived by adapting to new circumstances and demonstrating a willingness to change policies and adapt new procedures. Each of the events that have been described have occurred with vigorous debate. Although reasonable solutions were often met with great difficulty, research has proceeded in a socially responsible manner. He stated that it is his hope that, through a process of honest and respectful discussion and debate, difficult problems will continue to be resolved.

III. REMARKS OF THE DIRECTOR OF NIH--DR. BERNADINE HEALY

Dr. Healy explained that she requested this special session of the RAC to discuss the NIH approval of Dr. Royston's compassionate plea exemption request and to address the urgent need to establish guidelines for handling such requests in the future.

Dr. Healy presented a chronology of the events that led to her decision to approve Dr. Royston's request. In early October, Senator Tom Harkin asked that the NIH *give timely consideration to individual compassionate plea requests for approval of gene therapy procedures for terminally ill patients. Such a step would be a temporary solution to this problem until an appropriate, permanent, legislative solution setting forth the clear authority and mechanism for handling these cases can be achieved next year.* This request was made on behalf of a 51-year-old former constituent suffering from a life-threatening Stage IV glioblastoma that has been unresponsive to conventional treatments.

At the time NIH received Senator Harkin's letter, there was insufficient information to make a decision regarding this particular patient or the proposed treatment. This request was the first compassionate plea for gene therapy received at NIH, and the NIH has no experience or mechanism for addressing such pleas. It was imperative that the RAC consider this issue at the December 4, 1992, meeting. Accordingly, Dr. Royston was invited to attend the December 1992 meeting to provide further information. Dr. Healy stated that although the RAC had a lengthy discussion on this issue, a formal recommendation concerning either the specific patient or the generic issue of compassionate plea exemptions was not forthcoming. Subsequently, Dr. Royston has submitted a formal written request for compassionate plea exemption to the NIH, including a copy of his Food and Drug Administration (FDA) single patient Investigational New Drug (IND) application and protocol, which were already pending at the FDA and his Institutional Review Board (IRB).

Dr. Lance Liotta, Deputy Director for Intramural Research, NIH, reviewed Dr. Royston's materials with the assistance of several oncologists at the National Cancer Institute (NCI), the staff of the Office of Human Subjects Research, and Dr. Wivel, Director of the Office of Recombinant DNA Activities (ORDA).

Consistent with the RAC's discussion on December 4, 1992, a letter was sent to Dr. Royston on December 21, 1992, recommending that his patient should consider entry into Dr. Edward Oldfield's glioblastoma protocol. At this time, Dr. Liotta requested additional information regarding local approvals and the status of the patient. Within 24 hours of this recommendation, NIH was advised that the patient and her family declined to be considered for Dr. Oldfield's protocol, in part because of the requirement for additional surgery. Moreover, this patient has already undergone several craniotomies that have probably caused her to be ineligible for Dr. Oldfield's protocol. In contrast,

Dr. Royston's protocol is minimally invasive, involving the peripheral subcutaneous injection of gene-modified cells.

On December 23, 1992, Dr. Liotta, Dr. Wivel, and Mr. Lanman (NIH's legal counsel) met with the staff of the Assistant Secretary for Health and representatives of the FDA. At that meeting, the FDA representatives announced that Dr. Royston's single patient IND for compassionate use of gene therapy would probably be granted. In light of this announcement, Mr. Lanman advised NIH that absent any mechanism for compassionate plea exemption in the *NIH Guidelines*, it is within the authority of the NIH Director to make a decision regarding this request, pending FDA approval.

On December 28, 1992, the FDA advised NIH in writing that Dr. Royston's IND request had been approved with the use of Dr. Bernd Gansbacher's vector, NAPAD/IL2. Dr. Gansbacher had previously received NIH and FDA approval for the use of this vector in melanoma and renal cell carcinoma trials. The FDA viewed Dr. Royston's protocol as an extension of Dr. Gansbacher's protocol. Dr. Healy stated that based on FDA approval and concurrence from Dr. Liotta, Mr. Lanman, and several NIH institute and division directors, she approved Dr. Royston's compassionate plea request. Subsequently, this action was endorsed unanimously by the NIH institute and center directors.

Dr. Healy stated that her decision was based on the grave condition of the patient, the failure of all other available therapies, and an estimated survival of less than two months. First and foremost, the decision was a compassionate response to the request of a dying patient. This action was taken only after the assurance that there was no significant risk to either the health of the patient or the public.

Dr. Healy reminded the RAC that this meeting was not held for the purpose of critiquing this action. Rather, the RAC should consider with some urgency the development of a process for considering compassionate plea requests for gene therapy in the future. During the December 1992 meeting, one element was missing from the RAC's discussion; namely, the element of concern for an individual patient and their family. The evolution of genetic research now imposes this element upon us. Given the serious plight of Dr. Royston's patient, the following two alternatives offered by the RAC were unacceptable: (1) inclusion of the patient in Dr. Oldfield's protocol, and (2) continued deliberations at the March 1-2, 1993, RAC meeting.

Dr. Healy stated that there are at least three deficiencies in the current system for dealing with this new frontier of human gene therapy. First, the specific issue of compassionate use exemption is not encompassed by the *NIH Guidelines*, which embrace only research involving the use of recombinant DNA. Therefore, the argument can be made that a non-research compassionate plea exemption for a single patient is outside

the scope of the RAC mandate. Second, should the *NIH Guidelines* be amended such that non-research exemptions fall within the purview of the RAC? The letter of the *NIH Guidelines* might support the exclusion of such cases, but the spirit of the RAC mandate, to protect the public and provide a process for public comment on recombinant DNA research, might argue otherwise. The *NIH Guidelines* states that, *The NIH Guidelines cannot anticipate every possible situation. The Guidelines will never be complete or final, since all conceivable experiments involving recombinant DNA cannot be foreseen. Therefore, it is the responsibility of the institution and those associated with it to adhere to the intent of the Guidelines as well as to their specifics.* This statement suggests that the NIH's broader mandate is to clarify the *NIH Guidelines* as science evolves. Third, absent any mechanism and faced with urgent requests for the use of gene therapy in imminently dying patients, an NIH interim policy must be in place while this issue is thoroughly reviewed and long-term options are developed. Proposed policy regarding this issue should be developed based on input from the members of the RAC, the scientific research community, in coordination with FDA procedures and statutory authorities, and full public dialogue.

Dr. Healy stated that this meeting serves two purposes: (1) to inform the RAC of the interim approach that has been put into place to handle Dr. Royston's request expeditiously; and (2) to develop formal procedures for addressing future compassionate plea requests.

Dr. Healy outlined the interim approach that will be taken by the NIH absent a formal procedure: (1) If an experimental recombinant DNA product proposed for compassionate therapeutic use is derived from research that is subject to the *NIH Guidelines*, NIH has a responsibility to respond to that request. (2) This response must be made in a timely manner consistent with existing practices for compassionate use exemptions for other drugs and biologics. To the extent consistent with the need for rapid action, public notification will occur. In any case, the decision and the basis for the decision will be made public. (3) NIH will gather appropriate information on patient and public safety, including all documents submitted to other review bodies. NIH will seek advice of the appropriate Institute, Center, and Division representatives; other experts; the RAC Chair; and other individuals deemed necessary by the Chair. (4) NIH approval of a compassionate plea request will be contingent on FDA, IRB, and Institutional Biosafety Committee (IBC) approvals.

Historically, the strength of the RAC is that it has always been responsible to the changing nature of science and public concerns. The submission of a compassionate plea request was inevitable. The promise of gene therapy is too great; the needs of the patient community are too desperate.

Dr. Healy stated that in the context of this pressing matter, the RAC is charged with developing recommendations that will provide a long-term procedure for handling compassionate plea requests for gene therapy. In executing this charge, the RAC needs to consider the following questions: (1) What should the scope of the RAC's role be in reviewing non-research compassionate plea requests for gene therapy? (2) How will the circumstances under which a non-research compassionate plea request for NIH approval be defined? (3) Does the RAC, as currently constituted, have adequate expertise to review individual patient requests? (4) How can a time-sensitive mechanism be developed for handling these requests that involves the public? This response must balance the need for public information with the needs of the patient for privacy and timely review. (5) How should the RAC mandate be expanded or enhanced to accommodate this new responsibility?

Dr. Healy concluded her remarks noting that the evolution of the *NIH Guidelines* must reflect the NIH's mission to apply scientific knowledge in order to extend the health of human lives and to reduce the burden resulting from their disease and disability. It is in this spirit that the RAC is asked to undertake this task.

IV. COMMITTEE'S DISCUSSION

Dr. Walters asked if the RAC members' had any questions and comments for Dr. Healy. The committee should focus their discussion on the five questions outlined by Dr. Healy. Dr. Walters recognized the RAC members who were joining the discussion via conference call, namely, Mr. Capron, Dr. Geiduschek, and Dr. Schaechter. Dr. Walters noted the order in which speakers would be recognized. The RAC members will have the first opportunity to respond, followed by liaison representatives, other NIH employees, members of the public who have submitted a formal request to address the committee, followed by the public at large.

Dr. Parkman said that many of the RAC members have had extensive experience with the expedited approval or compassionate use process. He inquired whether compassionate use implies the probability of efficacy. Specifically, is a Phase I study a potential topic for compassionate use? There is a potential ethical and societal difference between therapy that has the probability of efficacy and the rights of all people to have access to that therapy, regardless if the intent is purely experimental.

Dr. Parkman explained that the process for determining the answers to these Phase I compassionate use questions is already in place at the level of the IRB. Many IRBs have a ruling that a compassionate use protocol that presents significant risk has to be reviewed by the full body, whereas a protocol that provides minimal risk can be administratively approved by the IRB Chair using any assistance necessary. It is important that the RAC adopt the same criteria for the assessment of compassionate use

protocols for human gene therapy. In addition, the use of the compassionate use mechanism should be granted solely on a one-time basis.

Dr. Krogstad stated that in terms of public perception, the words *compassionate use* suggests a reasonable expectation of efficacy. During the course of this discussion, the RAC should consider the public's perception. It is unfortunate that Dr. Royston's protocol has brought the issue of compassionate use of gene therapy to the RAC, because this protocol has serious scientific and medical shortcomings. However, Dr. Krogstad supported Dr. Healy's effort to develop a mechanism for the approval of compassionate plea requests.

Dr. Leventhal said that the RAC should discuss whether any patient who is treated under a compassionate use exemption should be considered a research subject. The Office for Protection from Research Risk maintains that such patients cannot be considered research subjects. An investigator who receives a single patient compassionate use exemption cannot include the results of that patient data in any further reports of their research. Considering the public nature of this committee and the great interest in gene therapy, it is going to be extremely difficult not to interpret results.

Dr. Zallen noted the NIH has been particularly concerned about the accessibility of women and minorities to research protocols. She asked Dr. Healy if these same concerns will be applied to compassionate use policies. Dr. Healy stated that compassionate use exemptions are granted on a case-by-case basis. These exemptions are non-research. Although all U.S. citizens should have equal and fair access to research protocols, compassionate use cannot be considered research. Data regarding compassionate use patients may not be part of data that is presented as a generalizable body of knowledge, which is explicitly stated in existing NIH regulations.

Dr. Walters recognized the NIH staff who were present at the table: Mr. Robert Lanman, NIH Legal Counsel; Ms. Sandy Chamblee, Senior Policy Advisor and Counselor to the Director; Dr. John Diggs, Deputy Director for Extramural Research; Dr. John Mahoney, Associate Director for Administration; Dr. Lance Liotta, Deputy Director for Intramural Research; and Dr. Bruce Chabner, Director of the Division of Cancer Treatment, NCI.

Dr. Chabner responded to the issue of efficacy of compassionate use therapy. Safety should be the important consideration, not efficacy. NCI's Division of Cancer Treatment handles numerous compassionate use requests; in many instances, there is very little, if any, information to suggest that the therapy will be effective.

Ms. Buc said that she had several suggestions for separating the RAC's role from NIH's role on this issue. During the course of this discussion, the RAC must consider the role of an advisory committee versus the government. Advisory committees advise and governments govern. The RAC serves an enormously important purpose of exploring, discussing, and providing a wide variety of expertise. But as its name states, the RAC is simply an advisory committee to the NIH Director. The NIH Director has the authority and responsibility to decide on each protocol that is presented to the RAC.

Ms. Buc stated that Dr. Healy had ample legal authority to make this decision. Perhaps more important, she attempted to include the RAC and other individuals in the process. Dr. Healy should exercise her authority to grant compassionate use exemptions for gene therapy, consult the RAC if possible, and notify the public by an appropriate mechanism. Ms. Buc suggested that discussion should focus towards reducing the RAC's formal exposure to compassionate use requests; however, the committee should assist NIH in establishing guidelines for the consideration of such requests.

Dr. Chase stated that he respected the process by which Dr. Healy made her decision regarding Dr. Royston's compassionate plea request. Dr. Chase said that in his opinion, the December 1992 RAC meeting would have been more productive if Dr. Healy had been present. The RAC would have attempted to support Dr. Healy's original position that Dr. Royston's request should not be granted because of both inadequate safety review and the lack of demonstrable evidence that the treatment would be efficacious.

Dr. Healy said that when Dr. Royston's patient was originally brought to NIH's attention in October 1992, no safety data had been obtained. This safety documentation was submitted in December 1992, around the time of the RAC meeting. Dr. Healy stated that her decision to grant this request was based on recent safety data and the advice of NIH staff. Safety was the overriding issue that governed the FDA's deliberations. This informed judgement was based on new information submitted to the NIH and the FDA.

Dr. Walters instructed the RAC members and other NIH officials to examine the 3-page proposal that was developed by the working group on compassionate plea exemptions. Dr. Walters outlined the working group's proposal. The introductory statement of this document explains that the ideal submission and review for compassionate use requests, including single patient protocols, is eight weeks in advance of a scheduled RAC meeting. However, in the event that this time-frame cannot be maintained, the working group proposed the following options: (1) Expedited RAC review, either special meetings between regularly scheduled RAC meetings, or some similar mechanism for reviewing compassionate plea requests that arise between meetings where emergency action is needed. (2) Referral to previously approved protocols; however, the RAC must consider the situation where a protocol already has a long waiting list of patients who meet the appropriate inclusion/exclusion criteria. (3) Allow new investigators to

use vectors that have already been reviewed and approved by the RAC for human gene therapy. (4) Consider compassionate use requests outside of the research system and require that data from such treatments cannot be used in research publications. In conclusion, the proposal states that the working group does not recommend any of these alternative mechanisms; because there are serious reservations about each point. The working group has outlined these items so that the entire RAC and Dr. Healy could refine a particular option or propose an entirely new alternative.

Dr. Post said that he agreed with the statement made by Ms. Buc regarding the authority of the NIH Director to make these decisions. Since the RAC does not readily fit into the compassionate plea request process, perhaps a final alternative should be that the RAC should not entertain these requests.

Ms. Buc stated that she was puzzled by point number 4. There seems to be some kind of punishment intended for investigators using compassionate plea requests; namely, they do not have the opportunity to cite their results. Denying publication to these investigators undercuts an important aspect of our effort; namely, deriving important information from these early experiments. The FDA recognizes that compassionate use requests are not full-blown research; however, their regulations require that investigators must report any information obtained as a result of the procedure. Therefore, the NIH and the RAC should not allow investigators to relinquish their obligation to file a report.

Dr. Healy reminded Ms. Buc that investigators always have the option of publishing a case report. This mechanism allows the investigator to relay new information, but it does not require the statistical validity of a research publication. Dr. Healy stated that compassionate use treatments can be certainly considered valid as case reports.

Dr. Healy reminded the RAC that her comments regarding the non-research component of compassionate use requests were not intended to get the RAC *off-the-hook*. The RAC should have a role if an experimental therapy is a direct derivative of research conducted and supported by the NIH. The NIH needs the advice of the RAC. How can a system be created in which the RAC can provide advice and balance the needs of a patient that is in imminent danger? In turn, if a patient is not in imminent danger, then the proposal can wait until the next scheduled RAC meeting. Dr. Healy said she has learned during this process that the RAC cannot convene without the appropriate public notice. These are uncharted waters. The flexibility of the RAC is important. If NIH cannot depend on the advice of the RAC then it will have to seek the advice of some other similar body. Dr. Healy stated that advice regarding gene therapy should come from the RAC.

Mr. Capron inquired how the NIH understands the basis for compassionate use exemptions granted by the FDA. Is there any expectation that other review processes

would be required, i.e., IRB and IBC review? Dr. Healy responded that the FDA includes the standard review procedures as part of their compassionate use IND process. Dr. Healy reminded Mr. Capron that the NIH did not deliberate with the FDA. The FDA conducted an independent review using the standard procedures for compassionate use single patient INDs. NIH did not make a decision regarding this issue until the FDA had granted approval, because the FDA has regulatory authority and statutory authority. The NIH used the FDA's approval to help guide its decision.

Mr. Capron said that he understands FDA has a process, the question is what is the basis for this process? If the RAC develops a compassionate use process as has been suggested, would the FDA become the only real review mechanism? If an investigator is faced with a lengthy 8-week review mechanism with public scrutiny or the closed-session quick review provided by the FDA, what course would investigator's choose? Mr. Capron stated that he could not imagine that there would be many investigators that would choose the long review process. Maybe the committee should face the judgement that the RAC approval process is in *the evolution of the field*, as Dr. Healy has stated; and it is no longer necessary. The RAC will no longer be relied upon. At the very least, it would be relied upon in those cases where the research is most justified; where the design of the research and the preclinical work has been fully carried out and is most defensible. In the least defensible cases where those protocols could not pass the RAC's scrutiny, the alternative approach with less public process will be chosen. Mr. Capron said that it is his understanding that Dr. Royston's IBC never granted approval for this compassionate plea protocol. The RAC has not seen the data that was presented to the FDA.

Dr. Healy reminded Mr. Capron that Dr. Royston's request was not viewed as a research proposal. NIH did not grant approval for research to be performed that was not believed to have been adequately reviewed as a research protocol by the RAC. Dr. Healy explained that the phrase, *evolution of the field*, does not imply that the field has become so advanced that the RAC is no longer needed. This phrase refers to the evolution of human gene therapy, expectations about therapies, and evolution to the patient. Dr. Healy said that she is deeply concerned about the notion that the FDA should be the sole decider of compassionate use exemptions that are derived from research that would otherwise come under the purview of the RAC. There might be circumstances in which the FDA would approve a compassionate use IND, and NIH would deny approval because of concerns about the vector or other safety aspect of the procedure.

Dr. Healy explained that the NIH spent a great deal of time reviewing the safety issues surrounding this particular vector. Drs. Liotta, Chabner, Broder, and others, reviewed Dr. Royston's request repeatedly and the questions were: Are there safety issues? Is there something unique? Is this protocol similar to Dr. Gansbacher's protocol? Does

the NIH have a basis upon which we would disagree with FDA's conclusion? Upon consideration of these questions, NIH could find no basis for determining that the FDA had made a mistake in granting this approval. However, it would be a mistake for the NIH to state that it would defer all compassionate use requests for gene therapy to the FDA just because NIH agreed with the FDA's decision in this case. It would be tragic for the RAC to state that because of one exemption, the committee is no longer needed. The RAC is needed now more than ever as expanded human therapies are being explored.

Dr. Leventhal said that she would like to discuss the use of the word *compassionate* as it implies to the patient's need for this treatment. The FDA term *single patient use* is a more preferable description of this procedure. The RAC should protect patients from feeling that they need a particular therapy that has never been proven to provide clinical benefit.

Dr. Krogstad said that he has trouble separating the development of compassionate use procedures from the specific case at hand. It is important to clarify that the circumstances surrounding Dr. Royston's request were created when the original protocol was rejected by the RAC as being inadequate on both scientific and medical grounds. This situation should not erupt again.

Dr. Parkman asked Dr. Chabner if it is appropriate for the first person to receive a novel therapy to be on a compassionate use basis, i.e., there is no supporting data regarding the safety of that therapy in human?

Dr. Parkman reiterated his view that there is no mechanism outside of the current 3 month meeting cycle that allows the RAC to have a formal advisory role. The rules can be changed to allow for expedited review, but that would make the review process private instead of public. The RAC must balance the right of the individual to receive expedited review with the right of the public to be informed about these proposals. Dr. Wivel stated that Dr. Parkman has addressed the essential point; specifically, the *NIH Guidelines* require that the RAC is under obligation to conduct its discussions in public. Notice of meeting has to be given in consideration of the Federal Advisory Committee Act. The public must be allowed to comment on the agenda, and the meeting materials are made public. In the event that the RAC chooses to modify the procedures as they currently exist, they would have to decide if the public should be notified in precisely the same manner as in the past or decide whether gene therapy has reached the level of acceptance that it is no longer viewed as a threat to the safety of the public.

Dr. Chabner responded to Dr. Parkman's question. Dr. Chabner explained that there is always a first patient to receive every therapy, and this usually occurs within the context of an experimental protocol. However, there are thousands of patients who receive

approval for the compassionate use of drugs under much different circumstances. Drug use in this country is not necessarily regulated on the basis of efficacy in experimental situations or in compassionate use situations. For example, there is widespread use of a variety of drugs for the treatment of acquired immunodeficiency syndrome (AIDS) that have not been proven to be efficacious in the treatment of this disease. A balance has been struck between the right of an individual patient to have access to an experimental drug or therapy and the concerns of the community about safety. Dr. Healy's decision is consistent with the way that compassionate drug use is being used for diseases such as AIDS.

V. FDA STATEMENT--DR. WOODCOCK

Dr. Janet Woodcock, Director of the Office of Therapeutics of the Center for Biologics Evaluation and Research, stated her intention to clarify some of the questions that have arisen during the course of this discussion regarding the FDA's review of gene therapy. The vectors that are used for gene therapy are classified as investigational biological products. Therefore, vectors used for human gene therapy come under statutory authority for regulating investigational products, investigational drugs, and biologics. The FDA has a defined set of regulations that are followed for the approval of human protocols. A 2-page FDA document was distributed summarizing single patient use and emergency use situations.

Dr. Woodcock stated that the RAC plays an important complementary role to the FDA. The FDA focuses on manufacturing, i.e., the production of these experimental biologicals. The FDA does not always provide the opportunity for open public discussion that the RAC permits.

The FDA's review of any investigation protocol in humans includes an assurance by the investigator and an understanding that any applicable regulations, policies, or approvals, have been obtained and followed including IRB, IBC, and RAC approval, if appropriate.

There has been increasing pressure from patients, patient advocacy groups, and the general public, to increase access to experimental therapies earlier in the investigational stages. Traditionally, this access has been limited to drugs that were late in the development cycle. However, over the last 5 years, especially in human immunodeficiency virus related illnesses and cancer, there has been increasing pressure and response by the FDA to provide access to patients earlier in drug development. The FDA provides these therapies with the understanding that patients may be exposing themselves to the increased risk of toxicity. In addition, these patients may be deferring themselves from another more effective therapy that might be available.

Dr. Woodcock stated that the FDA encourages access to these drugs; however, the FDA discourages access before the agent has been tested in a Phase I trial because of uncertainties about the dose/toxicity profile of the drug. Many drugs that seem promising in the preclinical development phase are abandoned following a Phase I trial due to toxicity. However, the FDA still receives occasional requests for exceptions from patients who have exhausted all other therapies, do not fit the inclusion/exclusion criteria of the protocol, do not have geographic access to the protocol, or for other various reasons. In these rare cases, the FDA tries to work with the patient's physician and the investigator to provide some exception. Most of these cases are time-limited situations due to the life-threatening status of the patient.

Dr. Woodcock explained that the emergency use situation is more of a medical emergency in which there is insufficient time to perform the required paperwork. Physicians can call the FDA and request clearance for emergency use. Emergency use would ordinarily occur when there is some known effectiveness information about the agent, and there is a clear indication why the physician made the determination that the particular agent was needed.

VI. COMMITTEE'S DISCUSSION

Dr. Walters inquired as to the particular section of the FDA regulations that encompasses the single patient use category. Dr. Woodcock responded that the correct terminology is *single patient IND* which is found in section 312 of the 21 Code of Federal Regulations. An IND application exempts the sponsor from the general requirement to use an approved drug.

Dr. Parkman asked if the requirement for the submission of preclinical data is different for single patient protocols than for multiple patient protocols. Dr. Woodcock explained that virtually the same standards for submission data are required for both types of requests. However, the FDA statutory and regulatory regulations do not apply the same standards of preclinical rationale that the RAC requires.

As a point of clarification, Dr. Post inquired whether the FDA has granted first time use of investigational protocols on a single patient basis. Dr. Woodcock answered that the FDA has granted first time single patient use; however, these instances have been extremely rare.

Dr. Post explained that many of the RAC members are concerned that Dr. Royston's protocol was approved based on the documentation that was submitted to NIH. He asked Dr. Woodcock to provide the committee with qualitative information about the kinds of data that the FDA considered before granting approval of this request. Dr. Post stated that the members of the RAC would have more confidence in this decision if they

could be assured that Dr. Royston submitted significant documentation, other than the information sent to the NIH. Dr. Woodcock said that she could define the FDA's criteria for approving a gene therapy biologic for a Phase I trial. Dr. Post asked Dr. Woodcock to be as specific as possible about this particular approval. Dr. Woodcock said that she could not provide that information. Dr. Post asked Dr. Royston to respond to this question during his statement.

Dr. Woodcock stated that the vector proposed by Dr. Royston was already approved by the NIH and the FDA. Dr. Post said that the vector had been approved for use in Dr. Gansbacher's protocol. However, the cells have not been approved, which is an important distinction. Dr. Woodcock stated that any concerns the RAC would have about the cells probably focus on the activity and effectiveness of the cells rather than biosafety considerations. Dr. Post said that the safety of these cells is a primary consideration.

Dr. Woodcock stated that it is plausible that the RAC and the FDA would come to different conclusions. In fact, on every single gene therapy protocol the FDA has reviewed, there has been internal scientific disagreement. Dr. Royston's request is a sort of *hybrid* request because of the element of the cells. The investigational vector has been approved for a Phase I trial. However, the use of this vector was not simply an extension at a different geographic site. Obviously, Dr. Royston proposed to use the vector in a different manner than Dr. Gansbacher.

Dr. Geiduschek asked Dr. Woodcock if the FDA's approval of drugs that have never been approved for investigational use refers to the U.S. or anywhere in the world. Dr. Woodcock responded that these rare exemptions have been granted for drugs that have never been used in any Phase I trial in the world. However, the FDA is very discouraging about such uses, because there is no evidence of efficacy and no toxicity data is available on these agents.

Dr. Geiduschek asked for clarification regarding the criteria that the FDA uses to grant such approvals. Does the FDA grant compassionate plea exemptions for materials whose properties in humans are unknown? Dr. Woodcock responded that the FDA requires the same safety information whether the protocol is directed toward a specific patient or a total Phase I trial.

Dr. Haselkorn asked Dr. Woodcock if the FDA was provided any information on the safety of the transduced cells to be used in Dr. Royston's protocol. Dr. Woodcock said that the FDA outlined their requirements for safety testing previously, both for adventitious agent testing and for expression of the desired gene. Every lot of investigational biological agents administered to humans must meet a set of lot release criteria. These criteria are primarily safety and potency testing. Dr. Woodcock said that

she is not authorized to discuss this specific case in detail.

Dr. Leventhal inquired whether each autologous tumor cell preparation constitutes a lot. Dr. Woodcock said that the requirements for testing autologous cells are less stringent than for other biologics because of technical limitations.

Ms. Meyers stated that she is a public member on this committee who represents individuals with rare diseases, the majority of whom are as desperate as Dr. Royston's patient. She expressed concern about the criteria that the FDA uses to grant single patient INDs. Patients with AIDS and cancer are granted permission routinely to take drugs that are not approved and that are in the early stages of testing. However, the FDA stopped the production of a drug that was being produced by an investigator at Johns Hopkins University because of failure to pass *good manufacturing practices*. This drug, which was routinely manufactured for years, has kept hundreds of children alive who were diagnosed with a rare disease. Apparently, what is true for certain politically important groups is not true of the politically unimportant groups.

Ms. Meyers stated that her recommendation is that the RAC should send a letter, which includes recommendations on policy, to the incoming administration. There is no reason that the RAC should be forced into taking any action on this issue prior to January 20.

Ms. Meyers explained that there is an enormous difference between drugs and gene therapy. If a patient is adversely affected by a drug, only that patient is usually affected. With gene therapy, nothing is known about the long-term effects to the patient, health care workers, the patient's spouse, or the next generation.

Ms. Meyers explained that the Foundation for Economic Trends has gone to court in order to ensure that gene therapy deliberations are held in public, because society has a great stake in the outcome. However, the RAC is being urged to turn away from the public and allow these decisions to occur in private.

Ms. Meyers said that Dr. Royston's patient is not an inpatient. Therefore, she is allowed to go out into the public following treatment. The FDA approval of this protocol is analogous to approving an envelope without knowing the message of the letter. The members of the RAC are the experts that know what that message should be and the potential danger that can occur if that message is incorrect. The RAC was completely cut out of this approval process.

Ms. Meyers explained that Dr. Royston's protocol was deferred in 1991 because he was not prepared. The RAC told Dr. Royston to perform additional experiments. In 1991, Dr. Royston did not even have a patient in mind; he wanted to rush at that point. Dr. Royston said in November 1991 that he would resubmit his protocol in 3 months; he

never resubmitted the protocol. The RAC never heard from Dr. Royston until he returned to NIH with a request for a compassionate plea exemption. The RAC has still never received a complete submission. The information submitted to the NIH is completely unsatisfactory.

Ms. Meyers stated that the informed consent document that Dr. Royston's patient signed was untruthful. Dr. Royston has continually stated that, *this is the patient's last chance*, even though every piece of information submitted to the RAC indicates that this protocol will provide no therapeutic benefit. Dr. Royston's patient will not benefit from this treatment. The approval of this one compassionate plea request will open the door to thousands of similar requests, all asking to receive gene therapy that has not shown any effectiveness at all.

Dr. Chase said that there are aspects of this particular case that make consideration of the general problem difficult. These aspects are as follows: (1) there is the issue of equity of access; (2) there is a lack of public scrutiny regarding the ultimate decision process; (3) public involvement has been eliminated, which is an important principle underlying the function of the RAC; (3) there is a lack of scientific evidence supporting efficacy of the treatment; (4) there is the possibility that these extraordinary steps were taken in order to evade a more stringent review; and (5) there has been a derangement of normal Federal government processes.

VII. STATEMENT--DR. ROYSTON

Dr. Walters called on Dr. Royston to respond to the RAC members' questions and comments. Dr. Walters encouraged Dr. Royston to provide a productive and constructive interchange. Specifically, was there any information that was submitted to the NIH, the FDA, or both agencies, after the December 1992 RAC meeting that contributed to the decision-making process?

Dr. Royston stated that he was appalled by the comments that have been made by the members of the RAC. He said that he is trying to comply with every Federal regulation and the *NIH Guidelines*; there are no base motives. He said that he worked an entire weekend on the *Points to Consider*, only to be informed that approval had been granted by the NIH Director.

As a point of clarification, Dr. Royston said that the protocol submitted in November 1991 is different from this protocol. He stated that it is his intention to submit the revised gene therapy protocol to the RAC in 1993, as soon as the vectors are certified by the FDA. Dr. Royston stated that he does not want to rush to bring in a protocol, he wants to be methodical and careful.

In the interim, Dr. Royston said that he was able to transduce and grow this particular patient's glioblastoma tumor cells in culture. This procedure is no mean feat. The question was, can we help this patient? This patient is an individual with a Stage IV glioblastoma who has failed all conventional therapies. Therefore, the only therapy available to this patient is experimental therapy. It is right that she receive this treatment. This individual is a thriving woman who has a life expectancy of 1 to 2 months, who otherwise functions normally.

Dr. Royston stated that he is a physician and a medical researcher. He receives NIH grants; therefore, he does not intend on evading the RAC. However, if it comes to choosing between a physician and a researcher, he is a physician first. He wants to give his patient the best available therapy.

Dr. Royston explained that based on the scientific literature, there is a strong rationale for providing this patient with genetically modified, transduced glioblastoma cells. All of the additional data that the FDA requested was submitted. However, he is not prepared to present this additional data today. Dr. Royston said that he would present the scientific rationale for this study and all of the data submitted to the FDA at the March 1-2, 1993, RAC meeting. Dr. Royston commended Dr. Healy for granting approval of this compassionate use request.

Dr. Krogstad asked for clarification regarding the submission of additional material. Was additional material submitted to the FDA that was not submitted to the RAC? Dr. Royston acknowledged that this statement is correct. The following additional safety data was submitted to the FDA: (1) replication competent retrovirus data, (2) sterility data, (3) data on the transduced cell line, (4) data demonstrating the lack of contaminating helper virus, and (5) vector identity data.

Dr. Krogstad inquired how interleukin-2 (IL-2) production in transduced cells compared with the experimental data that the RAC reviewed. Dr. Royston stated that the RAC did not have access to the experimental data. Dr. Royston said that the transduced glioblastoma cells secrete 30 units of IL-2 per 2×10^6 cells per 24 hours.

Dr. Krogstad said that there is merit to having the RAC review the data on which this single case exemption was granted, even retrospectively, to satisfy the concerns that have arisen among the committee members. Dr. Royston explained that the RAC's concerns have to be weighed against the needs of individuals. Dr. Krogstad said that he understands these considerations, because he has been a physician for over 20 years. Dr. Krogstad said that the patient's interests must be paramount; however, there is no excuse for overlooking the need to review these other issues.

Dr. Royston said that he would provide the RAC with any data that it believes is

important to review. He does not want the RAC to accuse him of being evasive. Dr. Post said that he would like to take Dr. Royston up on his offer to provide the material that was submitted to the FDA which was never submitted to the RAC. Dr. Royston agreed to provide the FDA material for the March 1-2, 1993, RAC meeting.

Dr. Post stated that the RAC will make an exception to the January 4 submission deadline because the protocol is not under formal review. Submission of this additional information will suggest the mechanism that was used to approve this protocol. The record can be set straight, rather than continue the guesswork about what information was submitted to the FDA. Perhaps review of this additional information at the March 1993 meeting will evolve into a constructive process.

Dr. Geiduschek asked about the specific protocol that was reviewed by the San Diego Regional Cancer Center's IRB on December 18, 1992. Was it the transduced fibroblast protocol or the transduced glioblastoma tumor cell protocol? Dr. Royston responded that the IRB primarily reviewed the transduced glioblastoma protocol; however, it was requested that if the number of glioblastoma cells was insufficient, then fibroblasts could be used instead. Dr. Geiduschek asked if the material that was provided as part of the RAC mailing was the same material that was supplied to the IRB. Dr. Royston said that he could not recall if the same material was presented to the IRB. The protocol underwent several modifications.

Dr. Royston explained that the final protocol was never sent to the NIH. Dr. Geiduschek asked if the final protocol was substantially different from the original protocol. Dr. Royston said that it depends on whom you ask. The only significant difference is that the dose of radiation was lowered from 20,000 rads to 7,000 rads.

Dr. Royston said that the protocol that was submitted to NIH was written after returning from the December 1992 RAC meeting. At that meeting, the RAC said that they would entertain a single patient protocol. He stated that Dr. Wivel had requested that additional data should be submitted, including the *Points to Consider*, but was informed that the application could be withdrawn following Dr. Healy's approval. Dr. Royston said that he would supply this information for the March 1-2, 1993, RAC meeting.

Ms. Buc suggested that Dr. Royston should present the material that was submitted to the FDA at the March 1993 RAC meeting, not for formal review, but as a special presentation. Ms. Buc stated that she is extending a formal invitation to Dr. Royston to present this information at the March 1993 meeting. Ms. Buc stated that since Dr. Royston has accepted the invitation to return in March 1993, the RAC should turn to the question to how these compassionate plea requests will be handled in the future. Should these requests come to the RAC or a subset of the RAC? Should the *NIH Guidelines* be amended? These questions must be answered within the context of the Federal Advisory

Committee Act; however, Ms. Buc stated that this act may include provisions for emergency exceptions.

Ms. Buc said that the RAC must consider a number of questions. What constitutes an emergency exception or a single use exception? What are the criteria for these exceptions? What are the criteria that NIH should use in acting on these situations? What will the RAC's role be in this process? Should the number of RAC meetings increased? How should the NIH respond to a patient that does not fit into the criteria that will be established?

Ms. Buc stated that she was concerned about some of the statements that were made regarding the qualifications of the FDA and the RAC. The RAC is comprised of members who possess significant expertise. However, she stated that she has considerable confidence that the FDA can handle these compassionate requests on a single patient basis. It is unfair to say that only the RAC has the scientific competence to review a vector or a protocol.

Dr. Leventhal urged the RAC to defer any further discussion of the individual protocol; the remainder of the meeting should be dedicated to developing general policies on the issue of compassionate requests.

Dr. Carmen asked Dr. Royston how he would respond to the multitude of patients suffering from glioblastoma if they requested the same therapy that this woman received. Dr. Royston responded that he has already received approximately 100 requests, and that he referred these patients to Dr. Oldfield's protocol. Dr. Royston said that the NIH will now have to deal with compassionate requests for Dr. Oldfield's protocol.

Dr. Zallen noted that this compassionate request resulted from the rapid progression of this patient's disease. The RAC was informed that the 3 month period between meetings was too long for the patient to wait for treatment. However, the informed consent document is dated August 10, 1992. If Dr. Royston had requested RAC review of the protocol on this date, there would have been enough time to proceed through the standard RAC review process, i.e., at the December 1992 RAC meeting. Dr. Royston said that the reason for the delay was because of his misunderstanding regarding the availability of single patient protocol review by the RAC. Dr. Royston stated that a lot of things are easier in hindsight. In retrospect, the proposal probably should have been submitted as a single patient protocol before the deadline. Dr. Zallen explained that it is useful for the RAC to consider that had Dr. Royston been aware that there was a mechanism for the review of a single patient protocol, he could have met the submission criteria without the need for an alternative mechanism. Dr. Royston responded that if the committee had rejected the protocol on the basis of insufficient data and FDA

approval had been obtained, he would have urged the NIH Director to approve the request in any case.

VIII. COMMITTEE'S DISCUSSION

Dr. Krogstad stated that it is impossible to remove cells from a patient, grow them up, transduce them, and demonstrate their safety within 24 hours. Therefore, there should never be a need for the RAC to turn such a request around in 24 hours. If the investigator initiates the RAC review process at the time the cells are removed with the intent of transducing them, there always will be sufficient time to provide thorough review.

Dr. Parkman explained that RAC review of human gene therapy protocols generally evolves around three areas: the vector(s), the cells that will be transduced, and the laboratory in which the transduction will occur. To say that the same vector and the same cell type can be used in another investigator's laboratory is unacceptable. The RAC has never defined a protocol solely in terms of the subject(s). Although the RAC may choose to entertain single patient versus multiple patient protocols differently, the criteria should remain the same. If the RAC chooses to provide expedited review, then speed becomes synonymous with non-public review. The *NIH Guidelines* can be amended to accommodate non-public review; however, the RAC will become vulnerable to criticism from those individuals that believe strongly in public review of gene therapy. He stated that he is uncertain how the RAC should balance speed and efficiency against the public's rights to access.

Dr. Post commented that if the public component becomes non-existent, then should the RAC exist? Dr. Parkman stated that even if the RAC chooses to review single patient protocols differently from multiple patient protocols, the criteria for such review should remain constant even though the process for reviewing these proposals may differ.

Mr. Capron agreed with Dr. Parkman's description of the objectives; namely, to maintain competent standards. The RAC is a deliberative body which incorporates external review. This type of review is not easily adapted to the kind of process that Dr. Royston has suggested is necessary. Mr. Capron responded to the questions that were posed by Dr. Healy during her statement to the RAC: (1) What is the scope of the RAC's role in reviewing non-research compassionate pleas for gene therapy? Mr. Capron said that the expedited review process will have to rely on a different form of advice, which does not ensure public scrutiny. The design of the research that the RAC has traditionally been concerned about is irrelevant in a non-research situation. (2) What are the circumstances under which non-research compassionate pleas ought to require NIH approval? The NIH will have to determine the criteria for answering this question, not the RAC. (3) Does the RAC, as currently constituted, have adequate expertise to review

requests for individual patients involved in recombinant DNA intervention? The implication is that the RAC does not know how to review protocols that involve patients. There is an appropriate combination of expertise on the RAC. Individual patient requests differ from multiple patient requests only in that the design of the research and its potential to contribute to general knowledge is absent. (4) How can a time-sensitive mechanism be developed for handling such requests? Mr. Capron said that expedited review will probably have to come from the FDA and the NIH Director. (5) How can the RAC mandate be expanded or enhanced? Mr. Capron said that the results of these single patient protocols should be reported to the RAC. The RAC should have access to any data relating to safety or problems associated with the procedure.

Dr. Leventhal said that she was uncomfortable with the terminology *single patient protocol* because some of these protocols might be considered research. There may be genetic diseases so rare that there is only one person available in whom an important experiment can be performed. Therefore, single patient research protocols must be distinguishable from single patient non-research protocols. The RAC members have indicated that safety needs to be assured in either setting. However, in a non-research setting, a single patient may be given material without the strict review and the possibility of efficacy required for a research protocol. She stated that she would not accept the approval of any experiment in which the results were not reported to the RAC.

Dr. Geiduschek continued, stating that the RAC as a national public committee on human gene therapy, has a natural time limit. There will come a point in which the kind of review currently undertaken by the RAC will be deemed no longer necessary, and the committee will be abandoned. Until that time, the dual system of approval, namely, research versus non-research compassionate use, is not appropriate or in the public interest. Compassionate use should only be approved in the context of the working group's recommendations, i.e., expansion of the inclusion/exclusion criteria of a previously approved protocol. If the RAC chooses to grant compassionate use exemptions within the context of approved protocols, clear eligibility requirements, including the patient and the physician, would have to be established.

Dr. Geiduschek recommended that the retrospective examination of Dr. Royston's protocol will be helpful in bringing the RAC to an agreement on future policy. He agreed with the statement made by Mr. Capron; namely, that the qualifications of the RAC members are appropriate for the review of both single patient and multiple patient protocols.

Dr. Geiduschek asked for the opportunity to state his views before having to leave the telephone conference. He referred the RAC to his written statements and emphasized that these statements represented his best judgement at that moment, as they had in

advance of the meeting.

Dr. Parkman said that he disagrees with Dr. Healy's concept of a non-research protocol. Any patient that receives gene therapy will be treated at an institution that has an ongoing gene therapy research program. The issue is that there is a patient who intersects with those research interests and the time frame for instituting therapy does not fit within the time constraints of the RAC. Therefore, the term *single patient protocol* is appropriate to describe these situations. Although statistical evaluations will not be possible, statements can certainly be made regarding efficacy.

IX. STATEMENT--MS. KATHERINE MATTHEWS

Dr. Walters called on Ms. Katherine Matthews, staff attorney for biotechnology, of the Foundation on Economic Trends, to present her statement to the RAC. Ms. Matthews distributed a proposed amendment to the *NIH Guidelines* regarding the issue of compassionate plea requests. The Foundation on Economic Trends is concerned that as progress is being made in gene therapy, public review and scrutiny, is decreasing. The Foundation on Economic Trends represents the public interest. The particular case that has brought the issue of compassionate use before the RAC is outrageous; the worst possible case scenario. Undue political influence is very clearly present in this case.

Ms. Matthews stressed the importance for adopting an amendment to the *NIH Guidelines* that will prevent this problem from occurring in the future. The RAC needs to address the question of how the committee is going to deal with the reality that the NIH Director is a political appointee.

The proposed amendment to the *NIH Guidelines* that was distributed by Ms. Matthews could not be voted on at this meeting because it had not been published for public comment in the *Federal Register*. However, Ms. Matthews suggested that the RAC might choose to consider the proposed amendment at the March 1993 RAC meeting. Gene therapy is still in its infancy and does not have public understanding. The need to reassure the public that the process is being scrutinized by all sectors of society is critical.

Ms. Matthews stated that there would be grave concerns on the part of the Foundation on Economic Trends if gene therapy deliberations were to occur in a manner different from what is prescribed in the *NIH Guidelines*, namely, providing the opportunity for public comment.

Ms. Matthews outlined the following proposed amendments to the *NIH Guidelines*: (1) Section I-A reads, *...administration of gene therapy to human subjects...* will be amended to read, *...administration of gene therapy either to a group of human subjects or to a single human subject...*, (2) A new paragraph will be added to Section I-A to read, *...Any request*

for compassionate plea exemption to the procedures of these NIH Guidelines, see Section III-A, or request for any such emergency use of gene therapy in a human subject or subjects, shall be directed to the NIH RAC in the manner prescribed in these NIH Guidelines, see Section III-A, and shall comply with all public notice procedures.

Ms. Matthews stated that if there is any diminution of the public access to RAC deliberations in contravention of the existing law, the Foundation on Economic Trends would file a law suit against the NIH.

X. STATEMENT--DR. HENRY MILLER

Dr. Walters called on Dr. Henry Miller of the FDA to present his statement to the RAC. In the years since Asilomar, recombinant DNA has been defined and circumscribed as an area that requires special attention and regulation. Superimposing this additional regulation has introduced inevitable delays and regulatory disincentives for many activities involving recombinant DNA. There are essentially four layers of regulation for gene therapy: IBC approval, IRB approval, NIH approval, and FDA approval.

In much of the rest of the Federal government, there has been a move to stay away from imposing a separate, discreet, regulatory, paradigm for recombinant DNA. If Dr. Royston's compassionate request had been for a biologic other than gene therapy, the protocol would not have required evaluation by his IBC or the RAC. There may be subsets of gene therapy that may be relegated only to the usual, very comprehensive, very meticulous, methods of evaluation that can be accomplished by the local IRB and the FDA. The RAC should identify some of these rationally circumscribed subsets of gene therapy protocols.

XI. STATEMENT--HUSBAND OF DR. ROYSTON'S PATIENT

The husband of Dr. Royston's patient introduced himself to the members of the RAC. He added that he did not come to this meeting to be critical of the RAC or to try to bury it. As a point of clarification, the patient's husband stated that the informed consent document that was dated August 10, 1992, granted permission to Dr. Royston to grow his wife's tumor cells in culture. The informed consent document did not grant permission for the gene therapy protocol. His wife was granted a compassionate plea exemption, both by the FDA and the NIH. Therefore, he is not trying to obtain anything additional for her. What Dr. Healy did was not only thoughtful and compassionate, but clearly legal.

He stated that he is a lawyer and is quite knowledgeable in the biology of his wife's disease. He became deeply involved in his wife's therapy, i.e., radiation treatments, in January 1992. He was aware of the RAC through the *Federal Register* but was not able

to reach anyone at the NIH. He said that he is not suggesting that RAC meetings should not be open to the public. However, the RAC does not have to have the responsibility for compassionate plea exemptions if it does not choose to entertain these requests. Even if the RAC chooses to review compassionate plea requests, that authority can be taken away from the RAC.

Under the Federal Advisory Committee Act (as interpreted by *Dabney vs. Reagan* in 1992, in the Federal District Court of New York) the head of the agency who appoints an administrator does not have to call meetings. Therefore, the head of an agency that has an advisory committee does not have to call, listen, nor have put before that advisory committee, any matter of any kind whatsoever. He stated that his wife's request has turned into a turf battle.

There is one matter that he found particularly reprehensible; namely, the suggestion that there has been political interference with the RAC and its decision making process. For the record, he explained how Senator Harkin became involved in his wife's compassionate plea for gene therapy. He contacted a former friend and client asking for assistance. This friend said that he knew Senator Harkin from Iowa. The patient's husband informed his friend that his wife is from Iowa and that her family still lives in Iowa. In fact, his wife's sister-in-law worked on several of Senator Harkin's campaigns.

After the appropriate contacts were made, Senator Harkin sent a letter to Dr. Healy on October 8, 1992, requesting that his wife be considered for the compassionate use of gene therapy. This letter had been misconstrued as a threat to Dr. Healy that Senator Harkin would introduce pertinent legislation if the request was not acted upon. The patient's husband assured the RAC that Senator Harkin had not intended this letter as a threat; the Senator had merely offered his assistance. On October 20, 1992, Dr. Healy responded to Senator Harkin's letter stating that the RAC does not have a mechanism by which to consider individual compassionate plea exemption requests. The patient's husband stated that at this point there was insufficient time to submit a protocol within the 8-week time frame. Throughout this entire period, the FDA was extremely cooperative. He will always be grateful to the FDA for their cooperation. These are the facts surrounding any political involvement. He has never talked to Senator Harkin directly and, to the best of his knowledge, no one else with political influence has been involved in his wife's case.

He explained that between himself and his wife's doctors, contacts were made in Canada, England, Germany, France, Italy, and Japan. He has talked to individuals at 31 cancer centers and has spent over \$18,000 of his own money seeking treatment for his wife. His wife's tumor was identified on January 10, 1992. Her first resection was on January 14, 1992. She has received 36 radiation treatments, 16 magnetic resonance imagings, 5 computerized tomography scans, etc. On August 10, 1992, she had her entire right

temporal lobe removed. While Dr. Royston was attending the December 4, 1992, RAC meeting, the patient was informed that her tumor had increased in size by 30% in 2 weeks.

The patient's husband explained that he provided this background information regarding the course of his wife's disease to stress the importance of considering the time table involved in these crucial cases.

XII. STATEMENT--DR. ROBERT SOBOL

Dr. Robert Sobol of the San Diego Regional Cancer Center introduced himself as the co-principal investigator on this single patient gene therapy IND. He stated that he is here to share his perspectives regarding compassionate plea approvals in the hope that his views will be useful in formulating more effective RAC policies for gene therapy in the future.

Dr. Sobol said that he and Dr. Royston had hoped to follow the standard RAC and FDA review process; however, expeditious action was prompted by the deterioration of his patient's clinical condition. This patient has failed all conventional and experimental therapies. Dr. Sobol commended the FDA and Dr. Healy for their rapid responses to the compassionate plea request.

Dr. Sobol explained that the only reason that he did not obtain approval from the RAC is that the committee does not meet often enough. Currently, the portal of entry for Federally supported gene therapy protocols to the RAC is four times per year. These meetings are too infrequent. If the members of the RAC believe it is necessary to review all gene therapy protocols, then they must be prepared to meet more frequently to accommodate these patients. The RAC should also develop guidelines for the review of protocols that reflect modifications of previously approved studies.

XIII. COMMITTEE'S DISCUSSION

Dr. D. Miller asked Dr. Sobol why the RAC never received the *Points to Consider* responses or the materials that were submitted to the FDA. Dr. Sobol said that it was the investigators' understanding that this information was not germane to NIH's decision regarding the protocol. However, Dr. Sobol stated that the *Points to Consider* and the FDA submission material will be provided and discussed at the March 1993 RAC meeting.

Dr. Royston reminded Dr. D. Miller that it had never occurred to him that the RAC would entertain a single patient protocol. Dr. Royston explained that the *Points to Consider* responses were prepared in advance of receiving approval from the NIH

Director. Dr. Royston said that the *Points to Consider* responses are in his office.

Dr. Zallen noted that Dr. Royston has stated that if he had known it was possible to request RAC review of a single patient protocol, he would have made that request. Dr. Zallen asked Dr. Sobol if he believed that was the case. Dr. Sobol said that he did not feel that Dr. Royston's statement was true, given the time frame involved. Dr. Zallen reminded Dr. Sobol that he had obtained the patient's tissue in August of 1992. Dr. Sobol agreed that the patient's tissue was available, and it was their intention to request RAC review at that time. However, the patient's clinical condition deteriorated, and they were unaware that the RAC would review a single patient protocol. Dr. Sobol said that it is his understanding that the patient's clinical deterioration precluded submission of the protocol in time for consideration.

Dr. Royston stated that a significant amount of time elapsed between the time that the patient's cells were transduced and gene expression was demonstrated. Approval would not have been possible from the RAC in November 1992 because pertinent data was not available. Adequate data was not available until December 1992, at which time the December 4, 1992, meeting had already passed. Dr. Sobol acknowledged that the earlier conclusion, namely, that the investigators could have pulled the relevant information together in time for the December 4, 1992, meeting, was not a correct statement.

XIV. STATEMENT--DR. WILLIAMS

Dr. Walters called on Dr. R. Michael Williams to present his statement to the RAC. Dr. R. Michael Williams introduced himself as the Chairman of the Cancer Consulting Group, Professor of Medicine at Northwestern University, and Chief Medical Officer of the Cancer Treatment Centers of America. Dr. Williams explained that he is a practicing oncologist with a Ph.D. in immunology.

Dr. Williams stated that he is in support of Dr. Healy's decision to grant a compassionate permit for the use of gene therapy at the San Diego Regional Cancer Center. Dr. Williams said that he sees the devastation and the hopelessness that cancer brings to families every day. Dr. Williams stated that the RAC needs to be open to new ideas and respond to new therapies quickly. Every avenue and every possibility to offer hope and to save lives should be pursued.

Previous speculations, which raised fears about biological disasters emanating from gene research, have simply not come to pass. Discussions can and should continue, but people die every day from cancers that might be treatable by gene therapy-based techniques. Cancer patients have the right to be empowered as part of the treatment team that decided which therapy is right for them. Compassionate use of a therapy is just that, compassion.

Dr. Williams urged the RAC to exercise compassion in every case they examine. The committee should not adopt guidelines that will slow progress or block options. The RAC should not close any doors.

XV. COMMITTEE'S DISCUSSION

Dr. Walters called on Ms. Buc to propose a motion in an attempt to move the RAC towards the adoption of a policy statement regarding expedited review of time-sensitive gene therapy protocols. Ms. Buc said that many comments have been made throughout the day by RAC members and others that can possibly be made into a common thread. The RAC is in agreement that it will consider single patient protocols; this issue is undisputed. She said that she would not attempt to make a distinction at this stage of gene therapy between research and therapy. Information can be obtained from these single patient protocols, even if there is no inherent statistical information resulting from these protocols.

Ms. Buc stated that the RAC is in agreement that the criteria are the same for single patient and multiple patient protocols, namely, the concern about safety. These criteria for safety and efficacy are flexible. If the therapy is apparently safe and the plight of the patient is desperate, individuals are more willing to grant approval. However, if safety is a concern and the patient is less desperate, the review may be more stringent.

Ms. Buc suggested that the NIH should publicly state that it has a very strong preference for the submission of protocols to the RAC; however, in emergency situations, NIH will review requests and apply the same criteria as the RAC. Obviously, such a public statement by the NIH will create a multitude of requests. NIH will have to make these determinations independently, applying the aforementioned criteria. The NIH should present a report at the next RAC meeting explaining what decisions have been made and the basis for those decisions. This report will allow for public participation and review. If the NIH's decisions are not a rough proxy of the decisions that would have been made by the RAC, then there will be divergence. If divergence occurs, public opinion will act to reduce that divergence in one way or another. In a sense, the RAC will act as peer review for NIH's decisions. In this way where the situation is critical, there is a safety valve.

Ms. Buc explained that as time passes, the RAC may decide to review fewer of these single patient protocols under some other more specific set of criteria, i.e., derivative of some other protocol. The NIH will handle compassionate plea requests; however, the RAC may choose to examine these decisions. The RAC should request that NIH agree in advance to provide a report following approval of these requests.

Committee Motion

Ms. Buc moved that the aforementioned recommendation is the consensus of the RAC. The motion was seconded by Dr. Parkman.

Ms. Meyers stated that she did not agree that any experiment should go forward without review and recommendation by the RAC. The RAC may recommend that a protocol should not be approved, and Dr. Healy will have the authority not to accept the RAC's advice. However, the protocol should still be reviewed by the RAC for the sake of public discussion. No protocol should be approved without RAC review. She stated that she had also been a member of the HGTS, which voted itself out of responsibility. Ms. Meyers said that everyone hopes that the RAC will eventually be unnecessary; however, the RAC is still needed to assure that the *NIH Guidelines* are followed. The need for public discussion of every single protocol is still necessary.

Dr. Krogstad addressed the issue of time. He recognized the fact that a patient's status can change radically within a short period of time; however, cells cannot be transduced, cultures defined, and assays performed within a matter of days. One possible approach would be for the investigator to notify the RAC at the time a patient is asked to sign the informed consent document granting permission to transduce their cells. The burden is placed on the investigators at the time they are considering transduction to notify the RAC.

Dr. Schaechter noted that he would not be able to remain on the telephone conference for the remainder of the meeting; however, he gave the RAC his proxy vote in favor of Ms. Buc's proposal.

Dr. Post said that he is in support of Ms. Buc's motion. It would be a very exceptional that approval of a protocol would happen before a RAC meeting. The proposed mechanism would still allow for public review, at most 2-3 months after the date that approval was granted. There would be a continual calibration of the RAC's standards versus the NIH Director's standards.

Ms. Buc explained that the real constraint on the RAC approval process is that the RAC is subject to a variety of statutes, guidelines, and regulations, that do not allow the committee to function in the same way that a government agency functions, i.e., to take executive action. The RAC has to deal with problems associated with establishing a quorum.

Ms. Meyers stated that the Federal courts have found that gene therapy must involve public disclosure. Ms. Buc said that the Federal courts have stated that advisory boards must function in public; however, there has never been a ruling regarding the necessity to seek the recommendations of an advisory board. The Federal government has the power to govern and to choose when it will seek the advice of an advisory committee.

Ms. Buc said that she is confident that the NIH Director will adopt the policy that she has proposed.

Dr. Parkman said that under the proposed policy, the only circumstance in which a protocol would be reviewed outside of the RAC is when there are time constraints that do not permit the normal review process. Therefore, the question is: who will review the protocol? Assuming that these requests are not too numerous, the NIH Director could choose to use RAC members as reviewers of these protocols rather than NIH staff. Using RAC members as reviewers would introduce consistency in the review process.

Ms. Meyers proposed an amendment to the motion. Upon announcement of this policy, the NIH Director will consult (if at all possible, both legally and practically) with current, former, and possible future members of the RAC.

Dr. Parkman said that one of the issues that could arise is the appropriateness of a protocol. There will be some protocols that, by the nature of the subject matter, are really not appropriate for expedited review. These decisions should closely tie to the procedures in effect at the IRB level.

Ms. Buc restated the amendment. The amendment is that the RAC recommends to the NIH Director that when this policy is announced, the Director will announce her intention to consult (to the extent legally and practically feasible) with the members of the RAC and others who can shed scientific, ethical, and legal guidance on the issue in a rapid manner. Dr. Moskowitz noted that this amendment is almost identical to the interim policy created by Dr. Healy and reminded the RAC that an attempt was made to contact Dr. Walters regarding Dr. Royston's approval. Dr. Parkman reminded Dr. Moskowitz that an attempt had been made to notify Dr. Walters that the decision had already been made, not to seek advice.

Dr. Carmen inquired about the legal standing of any vote that is taken by the RAC today. Dr. Walters responded that a quorum vote would be in favor of policy recommendation, which would go forward to the NIH Director.

In response to concerns raised by Dr. Carmen, Ms. Buc amended the policy statement that the NIH Director will take into account, among other factors, the degree of consanguinity to previously approved protocols. The further away the protocol is from a previously approved protocol, the greater the likelihood that it should be brought before the full RAC.

Dr. Carmen stated that the various reasons grounded in administrative law put forward by Dr. Healy's staff to support her decision to bypass RAC review in approving Dr. Royston's petition for expedited consideration were inadequate to sustain her disposition

of the case.

Dr. Leventhal said that she had several concerns. First, that every investigator who wants to have a clinical gene therapy protocol will be asking for permission on an emergency basis. That is the nature of clinical investigators. The proposed mechanism will only be adequate if the exceptions are rare. She also expressed concern about the additional burden this will create on the staff of the ORDA. Requests for compassionate use or single patient exemptions will overwhelm the routine business of the RAC. Dr. Wivel responded that it is his understanding that these requests would be reviewed by a group of individuals. These decisions will not be confined to ORDA, but will be reviewed by Dr. Liotta and other NIH staff.

Dr. Leventhal stated that there may be factors that lead the NIH Director to grant permission for a single patient to be treated that would not bear scrutiny when a full protocol is presented. The RAC must prepare themselves for the idea that just because the committee grants approval for one patient to be treated in a particular fashion, does not necessarily assume that the multiple patient protocol will be approved by the entire RAC.

Dr. DeLeon spoke in favor of the proposed policy recommendations. If the RAC is going to review these protocols *post facto* after NIH approval, then a report on the outcome of the trial should be submitted to the RAC. In addition, there should be a provision that protocols that have been disapproved by the RAC should not be submitted to the NIH Director as a way of circumventing the RAC.

Dr. Krogstad requested a friendly amendment to the policy statement. An investigator will notify ORDA at the time a patient's cells are transduced, which will ultimately be used for the purpose of gene therapy. The terms *compassionate use* or *compassionate therapy* should never be used. Ms. Buc asked if she could rephrase the amendment such that when the NIH is deciding whether a protocol is truly an emergency situation, the NIH should apprise itself of circumstances (i.e., intention to transduce the patient's cells) in order to assess whether there was ample time for the normal RAC review process. Dr. Krogstad agreed to Ms. Buc's revision of the amendment. This provision would avoid the submission of an emergency request from an investigator who transduced a patient's cells as long as 4 months prior to the request.

Dr. D. Miller stated that he would not vote in favor of the proposed policy statement. The *NIH Guidelines* are entirely suitable and should be strengthened, if anything, to include a provision for expedited review. He said that he does not have faith in the limited review that would be provided by the NIH or the FDA. Safety criteria might be relaxed to such an extent that a replication competent virus will cause lymphoproliferative disease in these patients.

Dr. D. Miller stated that he sides with the Foundation on Economic Trends with regard to the necessity for public review and scrutiny. There has never been a documented cure that is attributable to gene therapy. The RAC has reversed itself 180 degrees. Because of increasing pressure to approve trials for critically ill patients, investigators will attempt to administer anything to a patient in an effort to achieve some sort of an effect. To move in this direction is bad scientific policy. Progress will be hampered instead of approving good protocols quickly.

Dr. Woodcock asked to clarify a prevailing misconception about the FDA. The FDA's safety review of these protocols, like other investigational biologics, is extremely rigorous and includes a very detailed safety assessment of the cell line used to produce these products, the potential for adventitious agents contaminating the products, and any other safety risks that might occur. The FDA's safety evaluation is the same whether the protocol is intended for 1 or 100 patients.

Dr. Leventhal stated that due to the increasing number of human gene therapy protocols that are being submitted for RAC review, the committee might have to increase the number of meetings per year. She stated that increasing the number of meetings from 4 to 6 would decrease the time between meetings from 3 months to 2 months. Increasing the number of RAC meetings per year may resolve many of the problems discussed today.

Dr. Walters called on Dr. Wivel to summarize the proposed policy recommendation, including the amendments that were offered by the RAC members. Dr. Wivel outlined the policy statement as follows:

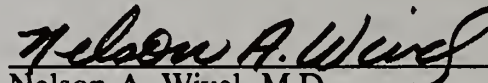
1. At the very outset, the RAC will make it clear that it will entertain single patient protocols.
2. No distinction will be made between research and therapy.
3. Regardless of the method of review, the criteria must be the same for all protocols.
4. NIH will state publicly as part of this policy statement, its preference for the traditional method of review.
5. When time sensitive circumstances prevail, the NIH will perform an internal review.
6. The NIH will take into consideration, among other factors, consanguinity with previously approved protocols.
7. To the extent legally and practically possible, the NIH Director will consult with RAC members, NIH experts, and any other experts deemed appropriate to the review process.
8. The NIH will report to the RAC following its internal review.

9. This policy statement will not include the terms *compassionate use* or *compassionate treatment*.
10. A protocol that has been deferred by the RAC in its normal review process will not be eligible for expedited review unless it is significantly altered from the original submission.
11. IRB and IBC approval are required prior to making a time-sensitive request from gene therapy to the NIH Director.
12. Any investigator who receives such an approval must report back to the RAC the results of the treatment.

The motion to approve this policy statement for recommendation to the NIH Director was approved by a vote of 9 in favor, 3 opposed, and 1 abstentions.

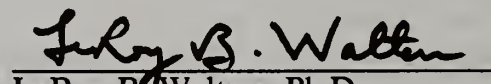
XVI. ADJOURNMENT

Dr. Walters adjourned the special meeting of the RAC at 3:52 p.m. on January 14, 1993.


Nelson A. Wivel, M.D.
Executive Secretary

I hereby acknowledge that, to the best of my knowledge, the foregoing Minutes and Attachment are accurate and complete.

Date: 3/1/93


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Recombinant DNA Research

Friday
February 12, 1993

Part VII

Department of Health and Human Services

National Institutes of Health

Recombinant DNA Advisory Committee;
Notice of Meeting and Proposed Actions;
Notices

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

Recombinant DNA Advisory Committee; Notice of Meeting

Pursuant to Public Law 92-463, notice is hereby given of a meeting of the Recombinant DNA Advisory Committee on March 1-2, 1993. The meeting will be held at the National Institutes of Health, Building 31C, Conference Room 6, 9000 Rockville Pike, Bethesda, Maryland 20892, starting at approximately 9 a.m. on March 1, 1993, to adjournment at approximately 5 p.m. on March 2, 1993. The meeting will be open to the public to discuss the following proposed actions under the NIH Guidelines for Research Involving Recombinant DNA Molecules (51 FR 16958):

- Proposed Major Actions to the NIH Guidelines;
- Additions to Appendix D of the NIH Guidelines regarding ten (10) Human Gene Therapy/Gene Transfer Protocols;
- Discussion regarding the Human Gene Therapy Protocol Materials submitted to the Food and Drug Administration for Approval on a Compassionate Plea Basis;
- Amendment to the Points to Consider in the Design and Submission of Protocols for the Transfer of Recombinant DNA into the Genome of Human Subjects Regarding the Use of Compassionate Plea;
- Amendment to the Points to Consider Regarding the Separation of the Gene Marking Informed Consent Document from the Therapeutic Informed Consent Document;
- Amendment to the Points to Consider Regarding Safety of Delivery/Expression Systems and Review Report on Murine Replication-Competent Retrovirus Assays; and
- Other Matters To Be Considered by the Committee.

Attendance by the public will be limited to space available. Members of the public wishing to speak at this meeting may be given such opportunity at the discretion of the Chair.

Dr. Nelson A. Wivel, Director, Office of Recombinant DNA Activities, National Institutes of Health, Building 31, room 4B11, Bethesda, Maryland 20892, Phone (301) 496-9838, FAX (301) 496-9839, will provide materials to be discussed at this meeting, roster of committee members, and substantive program information. Individuals who plan to attend and need special assistance, such as sign language

interpretation or other reasonable accommodations, should contact Dr. Wivel in advance of the meeting. A summary of the meeting will be available at a later date.

OMB's "Mandatory Information Requirements for Federal Assistance Program Announcements" (45 FR 39592, June 11, 1980) requires a statement concerning the official government programs contained in the Catalog of Federal Domestic Assistance. Normally NIH lists in its announcements the number and title of affected individual programs for the guidance of the public. Because the guidance in this notice covers not only virtually every NIH program but also essentially every Federal research program in which DNA recombinant molecule techniques could be used, it has been determined not to be cost effective or in the public interest of attempt to list these programs. Such a list would likely require several additional pages. In addition, NIH could not be certain that every Federal program would be included as many Federal agencies, as well as private organizations, both national and international, have elected to follow the NIH Guidelines. In lieu of the individual program listing, NIH invites readers to direct questions to the information address above about whether individual programs listed in the Catalog of Federal Domestic Assistance are affected.

Dated: February 8, 1993.

Susan K. Feldman,

Committee Management Officer, NIH.

[FR Doc. 93-3434 Filed 2-11-93; 8:45 am]

BILLING CODE 4140-01-M

Recombinant DNA Research; Proposed Actions Under the Guidelines

AGENCY: National Institutes of Health, PHS, DHHS.

ACTION: Notice of proposed actions under the NIH guidelines for research involving recombinant DNA molecules (51 FR 16958).

SUMMARY: This notice sets forth proposed actions to be taken under the National Institutes of Health (NIH) Guidelines for Research Involving Recombinant DNA Molecules (51 FR 16958). Interested parties are invited to submit comments concerning these proposals. These proposals will be considered by the Recombinant DNA Advisory Committee (RAC) at its meeting on March 1-2, 1993. After consideration of these proposals and comments by the RAC, the Director of

the National Institutes of Health will issue decisions in accordance with the NIH Guidelines.

DATES: Comments received by February 22, 1993, will be reproduced and distributed to the RAC for consideration at its March 1-2, 1993, meeting.

ADDRESSES: Written comments and recommendations should be submitted to Dr. Nelson A. Wivel, Director, Office of Recombinant DNA Activities (ORDA), Building 31, room 4B11, National Institutes of Health, Bethesda, Maryland 20892, or sent by FAX to 301-496-9839.

All comments received in timely response to this notice will be considered and will be considered and will be available for public inspection in the above office on weekdays between the hours of 8:30 a.m. and 5 p.m.

FOR FURTHER INFORMATION CONTACT:

Background documentation and additional information can be obtained from the Office of Recombinant DNA Activities, Building 31, room 4B11, National Institutes of Health, Bethesda, Maryland 20892, (301) 496-9838.

SUPPLEMENTARY INFORMATION: The NIH will consider the following actions under the NIH Guidelines for Research Involving Recombinant DNA Molecules:

I. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Therapy Protocol/Drs. Cassileth and Podack.

In a letter dated December 22, 1992, Drs. Peter A. Cassileth and Eckhard Podack, University of Miami, Miami, Florida, submitted a human gene therapy protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: Phase I Study of Transfected Cancer Cells Expressing the Interleukin-2 Gene Product in Limited Stage Small-Cell Lung Cancer.

II. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Therapy Protocol/Dr. Bank.

In a letter dated December 18, 1992, Dr. Arthur Bank, Columbia University, New York, New York, submitted a human gene therapy protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: Phase I Study of Gene Therapy for Breast Cancer.

III. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Therapy Protocol/Drs. Wilmott, Whitsett, Trapnell.

In a letter dated December 23, 1992, Drs. Robert W. Wilmott and Jeffrey Whitsett of Children's Hospital Medical Center, Cincinnati, Ohio, and Dr. Bruce Trapnell of Genetic Therapy, Inc., in Gaithersburg, Maryland, indicated the intention to submit a human gene

therapy protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: A Phase I Study of Gene Therapy of Cystic Fibrosis Utilizing a Replication Deficient Recombinant Adenovirus Vector to Deliver the Human Cystic Fibrosis Transmembrane Conductance Regulator cDNA to the Airways.

IV. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Therapy Protocol/Drs. Culver, Van Gilder.

In a letter dated November 7, 1992, Dr. Kenneth Culver of the Iowa Methodist Medical Center, Des Moines, Iowa, and Dr. John C. Van Gilder of the University of Iowa, Iowa City, Iowa, indicated the intention to submit a human gene therapy protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: Gene Therapy for the Treatment of Malignant Brain Tumors with In Vivo Tumor Transduction with the Herpes Simplex Thymidine Kinase Gene/Ganciclovir System.

V. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Therapy Protocol/Dr. Seigler.

In a letter dated December 16, 1992, Dr. Hilliard F. Seigler of the Duke University Medical Center, Durham, North Carolina, indicated the intention to submit a human gene therapy protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: A Phase I Trial of Human Gamma Interferon-Transduced Autologous Tumor Cells in Patients with Disseminated Malignant Melanoma.

VI. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Transfer Protocol/Drs. Brenner, Krance, Heslop, Santana, Ihle.

In a letter dated December 31, 1992, Drs. Malcolm Brenner, Robert Krance, Helen E. Heslop, Victor Santana, and James Ihle of the St. Jude's Children's Research Hospital, Memphis, Tennessee, submitted a human gene transfer protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: Assessment of the Efficacy of Purging by Using Gene-Marked Autologous Marrow Transplantation for Children with Acute Myelogenous Leukemia in First Complete Remission.

VII. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Therapy Protocol/Dr. Simons.

In a letter dated December 14, 1992, Dr. Jonathan Simons of the Johns Hopkins Oncology Center, Baltimore, Maryland, indicated the intention to

submit a human gene therapy protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: Phase I Study of Non-Replicating Autologous Tumor Cell Injections Using Cells Prepared With or Without Granulocyte-Macrophage Colony Stimulating Factor Gene Transduction in Patients with Metastatic Renal Cell Carcinoma.

VIII. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Therapy Protocol/Drs. Boucher, Knowles.

In a letter dated December 17, 1992, Drs. Richard C. Boucher and Michael R. Knowles of the University of North Carolina, Chapel Hill, North Carolina, indicated the intention to submit a human gene therapy protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: Gene Therapy for Cystic Fibrosis Using E1 Deleted Adenovirus: A Phase I Trial in the Nasal Cavity.

IX. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Transfer Protocol/Drs. Heslop, Brenner, Rooney.

In a letter dated December 31, 1992, Drs. Helen E. Heslop, Malcolm Brenner, and Cliona Rooney of the St. Jude Children's Research Hospital, Memphis, Tennessee, submitted a human gene transfer protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: Administration of Neomycin Resistance Gene Marked EBV Specific Cytotoxic T Lymphocytes to Recipients of Mismatched-Related or Phenotypically Similar Unrelated Donor Marrow Grafts.

X. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Therapy Protocol/Dr. Deisseroth.

In a letter dated January 4, 1993, Dr. Albert B. Deisseroth of the MD Anderson Cancer Center, University of Texas, Houston, Texas, submitted a human gene therapy protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: Use of Safety-Modified Retroviruses to Introduce Chemotherapy Resistance Sequences into Normal Hematopoietic Stem Cells for Chemoprotection During the Therapy of Ovarian Cancer.

XI. Discussion Regarding the Human Gene Therapy Protocol Materials submitted to the Food and Drug Administration for Approval on a Compassionate Plea Basis/Dr. Royston.

In a letter dated December 7, 1992, Dr. Ivor Royston, San Diego Regional Cancer Center, San Diego, California,

requested a compassionate plea approval for a human gene therapy protocol. The title of this protocol is: Phase I Study of Cytokine Therapy of Cancer, Active Immunotherapy of Glioblastoma with Tumor Cells or Fibroblasts Genetically Modified to Secrete Interleukin-2. On December 28, 1992, the Director, NIH, and the Food and Drug Administration granted protocol approval to Dr. Royston on a compassionate plea basis.

During the special Recombinant DNA Advisory Committee meeting on January 14, 1993, Dr. Royston stated that he would provide the committee with additional data that was supplied to the Food and Drug Administration. The materials included additional safety data on the transduced cell line, sterility data, data for replication competent virus, data looking for any evidence of contaminating helper virus, vector identity data, and data on production of Interleukin-2. Documents presented to the Institutional Review Board and the Institutional Biosafety Committee are to be submitted.

XII. Amendment to the "Points to Consider in the Design and Submission of Protocols for the Transfer of Recombinant DNA into the Genome of Human Subjects" Regarding the Use of Compassionate Plea.

The Points to Consider (March 1, 1990, 55 FR 7447) provide guidance to scientist and clinical investigators submitting human gene therapy/transfer protocols. During the Recombinant DNA Advisory Committee meeting on January 14, 1993, the committee adopted the following preliminary policy statement on approving human gene therapy protocols on an expedited basis for dying patients. This statement includes the following elements which are not listed in order of importance, but are simply meant to be inclusive of the issues that need to be addressed.

1. NIH will strongly emphasize that the standard method of protocol submission is highly preferred.

2. The RAC will consider single patient protocols.

3. There will be no attempt to distinguish between research and treatment in the consideration of protocols.

4. Regardless of the method of review, the criteria must be the same for all protocols.

5. When time-sensitive circumstances prevail, the NIH will do an internal review.

6. To the extent that it is legally and practically possible, the Director of NIH will ask NIH experts, RAC members, and other experts to participate in protocol review.

7. Among other factors to be considered by the Director of NIH, is the consanguinity of the new protocol to existing protocols.

8. The NIH will report to the RAC following its internal review.

9. Protocols that are deferred or not approved by the RAC in its normal review process, are not eligible for expedited review.

10. In the development of any documents that are a part of this policy statement, the terms, compassionate use and compassionate treatment, will be deliberately avoided.

The Recombinant DNA Advisory Committee will be further developing this policy statement for inclusion in the Points to Consider document.

XIII. Amendment to the Points to Consider Regarding Safety of Delivery/Expression Systems and Report on Murine Replication-Competent Retrovirus (RCR) Assays.

During the September 14-15, 1992, RAC meeting, there was a discussion regarding requirements for the assays of replication-competent retrovirus in vector supernatants. In the Points to Consider (March 1, 1990, 55 FR 7445), it states:

"1. Description of Proposal." * * *

"B. Research design, anticipated risks, and benefits." * * *

"2. Preclinical studies, including risk assessment studies." * * *

"c. Laboratory studies pertaining to the safety of the delivery/expression system.

"(1) If a retroviral system is used: * * *

"(b) How stable are the retroviral vector and the resulting provirus against loss, rearrangement, recombination, or mutation? What information is available on how much rearrangement or recombination with endogenous or other viral sequences is likely to occur in the patient's cells? What steps have been taken in designing the vector to minimize instability or variation? What laboratory studies have been performed to check for stability, and what is that sensitivity of the analyses? * * *

"(e) Has a protocol similar to the one proposed for a clinical trial been carried out in non-human primates and/or other animals? What were the results? Specifically, is there any evidence that the retroviral vector has recombined with any endogenous or other viral sequences in the animals?"

The recommended assays for detecting the presence of adventitious agents, including replication-competent retroviruses (RCR) have evolved as the RAC has gained experience in the review and approval of human gene transfer/therapy protocols. Recently, the Food and Drug Administration has been considering additional tests to increase the sensitivity of detection of RCR in vector supernatant preparations.

Since it is very important that retroviral vectors be free of RCR, it is important to quantitate the relative safety margin afforded by the assay systems used. To confirm that this safety margin is adequate, the RAC will discuss specific assay requirements and minimal levels of detection for possible inclusion in the Points to Consider.

During the Recombinant DNA Advisory Committee meeting on December 3-4, 1992, Drs. W. French Anderson, National Institutes of Health, Bethesda, Maryland; Gerard J. McGarrity, Genetic Therapy, Inc., Gaithersburg, Maryland; and Robert Moen, Genetic Therapy, Inc., Gaithersburg, Maryland, submitted a Report on Murine Replication-Competent Retrovirus (RCR) Assays. At the meeting, it was agreed that a modification of the report was warranted. The committee will be discussing the modified report during the March 1-2, 1993, meeting.

XIV. Amendment to the Points to Consider Regarding the Separation of the Gene Marking Informed Consent Document from the Therapeutic Informed Consent Documents.

During the September 14-15, 1992, RAC meeting, Dr. Leonard Post requested that when a gene transfer protocol is submitted as an addition to a therapeutic protocol, the principal

investigator should submit two separate informed consent documents, one for the gene marking portion and one for the therapeutic portion of the protocol. In the Points to Consider, Part I-D—Informed Consent (March 1, 1990, 55 FR 7446), a new sentence would be added to the introductory paragraph:

"When gene transfer is a procedure separate from the therapeutic protocol, an informed consent document should be submitted for both the gene marking and therapeutic procedures."

OMB's "Mandatory Information Requirements for Federal Assistance Program Announcements" (45 FR 39592, June 11, 1980) requires a statement concerning the official government programs contained in the Catalog of Federal Domestic Assistance. Normally, NIH lists in its announcements the number and title of affected individual programs for the guidance of the public. Because the guidance in this notice covers not only virtually every NIH program but also essentially every Federal research program in which DNA recombinant molecule techniques could be used, it has been determined not to be cost effective or in the public interest to attempt to list these programs. Such a list would likely require several additional pages. In addition, NIH could not be certain that every Federal program would be included at many Federal agencies, as well as private organizations, both national and international, have elected to follow the NIH Guidelines. In lieu of the individual program listing, NIH invites readers to direct questions to the information address above about whether individual programs listed in the Catalog of Federal Domestic Assistance are affected.

Dated: February 4, 1993.

Jay Moskowitz,

Associate Director for Science Policy and Legislation, NIH.

[FR Doc. 93-3433 Filed 2-11-93; 8:45 am]

BILLING CODE 4140-01-M

Thursday
February 18, 1993

Part VI

**Department of
Health and Human
Services**

National Institutes of Health

**Recombinant DNA Research: Actions
Under the Guidelines; Notice**

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

Recombinant DNA Research: Actions Under the Guidelines

AGENCY: National Institutes of Health, PHS, DHHS.

ACTION: Notice of Actions Under the NIH Guidelines for Research Involving Recombinant DNA Molecules.

SUMMARY: This notice sets forth seven actions to be taken by the Director, National Institutes of Health (NIH), under the May 7, 1986, NIH Guidelines for Research Involving Recombinant DNA Molecules (51 FR 16958).

FOR FURTHER INFORMATION CONTACT: Additional information can be obtained from Dr. Nelson A. Wivel, Director, Office of Recombinant DNA Activities (ORDA), Office of Science Policy and Legislation, National Institutes of Health, Building 31, room 4B11, Bethesda, Maryland 20892, (301) 496-9838.

SUPPLEMENTARY INFORMATION: Today seven actions are being promulgated under the NIH Guidelines for Research Involving Recombinant DNA Molecules. These seven proposed actions were published for comment in the Federal Register of January 3, 1992 (57 FR 316), May 6, 1992 (57 FR 19512), and August 19, 1992 (57 FR 37680), and reviewed and recommended for approval by the NIH Recombinant DNA Advisory Committee (RAC) at its meetings on February 10, 1992, June 1-2, 1992, and September 14-15, 1992.

I. Background Information and Decisions on Actions Under the NIH Guidelines

A. Addition to the Points to Consider in the Design and Submission of Protocols for the Transfer of Recombinant DNA into the Genome of Human Subjects Regarding Submission Requirements for Human Gene Transfer/Gene Therapy Protocols (March 1, 1990, 55 FR 7443)

Dr. Donald Krogstad, member of the RAC, suggested an outline for submission requirements for human gene transfer/gene therapy protocols during the RAC meeting of February 11, 1992. This outline reads as follows:

"Suggestions for Focusing Discussion at the RAC.

"I. Investigator-Submitted Material

"Format. Consider having both written and oral material presented in similar formats—e.g., following the *Points to Consider*—with a 150-250 word Abstract at the beginning of

written material. When a proposal has been presented previously, there should be a short section (≤150 words) detailing the major revisions since the previous submission.

"Length of Written Material and Time for Oral Presentation. Only on rare occasion is there significant new information after 20 pages of written material or 20 minutes of oral presentation. To expedite both written and oral review, written material could be limited to approximately 20 pages per proposal (not counting references, tables and figures), and oral presentations to 25 minutes.

"II. Reviewers' Responses

"Format. Reviewers' comments should state explicitly whether the *Points to Consider* have been addressed satisfactorily, and whether questions of Safety and Efficacy have been resolved. They should state also whether a proposal is:

"(a) Acceptable as written,
"(b) Acceptable with specific revisions, or

"(c) Unacceptable in its present form.

"Discussion of Written Reviews.

Discussion among the various reviewers and with the Principal Investigator should help to clarify substantive questions about the protocols, which currently occupies a substantial amount of time at the RAC.

"Length of Time for Oral Presentation of Reviews. After discussion with other reviewers and the Principal Investigator, it should be possible to present most reviews orally in ≤5 minutes."

This request was published for comment in the Federal Register of May 6, 1992 (57 FR 19512).

On June 2, 1992, the Committee reviewed and recommended approval of the following guidelines by a vote of 18 in favor, 0 opposed, and no abstentions.

"Guidelines for the Submission and Review of Human Gene Transfer/Therapy Protocols for Review by the Recombinant DNA Advisory Committee

"I. INVESTIGATOR-SUBMITTED MATERIAL:

"Written proposals should begin with the lay and scientific abstracts, followed by the *Points to Consider* and the material provided in the body of an R01 (sections A-D). When a proposal has been submitted previously, there should be a short section (≤200 words) immediately following the abstracts that summarizes major revisions since the last review. Length limitations are 4-5 pages for the *Points to Consider*, 2 pages each for CVs (Biosketch format) and 20 pages for the body of the proposal (excluding tables, figures, appendices,

and manuscripts). Data provided must include a description of the elements in the vector, the source of that information, and the method by which sequence data were compiled, plus 3 diskettes with the vector sequence in ASCII format. Written material from Principal Investigators must be submitted ≥8 weeks before the meeting at which it will be reviewed; written comments from the primary reviewers ≥4 weeks before the RAC meeting; and written responses from the Principal Investigators ≥2 weeks before the meeting.

"Oral Presentations at RAC Meetings provide only a brief overview of the proposal; they should concentrate on questions raised by the reviewers before and at the meeting. Oral presentations should be ≤20 minutes: ≤10 minutes for the overview and ≤10 minutes for responses to the reviewers' questions.

"II. Reviewers' Responses:

"Written Reviews should emphasize issues related to gene marking, gene transfer or gene therapy. They should state explicitly whether the *Points to Consider* have been addressed satisfactorily, and should examine the scientific rationale, scientific context (relative to other proposals reviewed by the RAC), whether the preliminary *in vitro* and *in vivo* data were obtained in appropriate models and are sufficient, and whether questions related to safety, efficacy, and social and ethical context have been resolved. Whenever possible, criticisms of Consent Forms should include suggested revisions for the RAC to consider—provided as written alternatives. Reviews should also state whether the proposal is:

"(a) Acceptable as written,

"(b) Acceptable with specific revisions or after satisfactory responses to specific questions raised on review, or

"(c) Unacceptable in its present form.

"Oral Discussion of Reviews at the RAC Meeting. It should be possible to present most reviews orally within ≤5 minutes."

I accept this recommendation and the *Points to Consider* of the NIH Guidelines will be amended accordingly.

B. Addition of Appendix D-XXXIV to the NIH Guidelines

In a letter dated May 1, 1992, Dr. Michael T. Lotze, University of Pittsburgh, Pittsburgh, Pennsylvania, indicated his intention to submit a human gene therapy protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: *Gene Therapy of Cancer: A Pilot Study of IL-4 Gene Modified Antitumor Vaccines*. This

request was published for comment in the *Federal Register* of August 19, 1992 (57 FR 37680).

The protocol was reviewed and recommended for approval during the RAC meeting on September 14-15, 1992, by a vote of 20 in favor, 0 opposed, and no abstentions, with the following stipulations: (1) Submission of the G11L4SvNa.25 vector sequence on a 3½ inch disk to be screened for open reading frames that code for unwanted gene products, and (2) the term "vaccine" should be deleted from the protocol and informed consent document.

On October 16, 1992, Dr. Lotze provided the information to ORDA. The data was reviewed by Drs. Leventhal, DeLeon, and Miller. Additional data was received from Dr. Lotze on December 10. After reviewing the data, it was determined that it meets the request of the RAC. The following section may be added to Appendix D: "Appendix D-XXXIV.

"Dr. Michael T. Lotze, University of Pittsburgh, Pittsburgh, Pennsylvania, can conduct experiments on twenty patients with metastatic, and/or unresectable, locally advanced melanoma, renal cell carcinoma, breast cancer, or colon cancer who have failed standard therapy. Patients will receive multiple subcutaneous injections of autologous tumor cells combined with an autologous fibroblast cell line that has been transduced *in vitro* with the gene coding for Interleukin-4 (IL-4) to augment the *in vivo* antitumor effect. Patients will be monitored for antitumor effect by PCR analysis and multiple biopsy of the injection site."

I accept this recommendation and Appendix D-XXXIV of the *NIH Guidelines* will be added accordingly.

C. Addition of Appendix D-XXXV to the *NIH Guidelines*

In a letter dated July 17, 1992, Dr. Friedrich G. Schuening, Fred Hutchinson Cancer Research Center, Seattle, Washington, submitted two human gene transfer protocols to the Recombinant DNA Advisory Committee for formal review and approval. The titles of these protocols are: (1) *Phase I/II Study of the Use of Recombinant Human Interleukin 3 (rhIL3) Stimulated Peripheral Blood Progenitor Cell Supplementation in Autologous Bone Marrow Transplantation in Patients with Breast Carcinoma or Hodgkin's Disease*, and (2) *Evaluation of the Use of Recombinant Human G-CSF Stimulated Peripheral Blood Progenitor Cell Supplementation in Autologous Bone Marrow Transplantation in Patients with Lymphoid Malignancies*.

This request was published for comment in the *Federal Register* of August 19, 1992 (57 FR 37680).

These protocols were reviewed and recommended for approval during the RAC meeting on September 14-15, 1992, by a vote of 12 in favor, 4 opposed, and 1 abstention, with the notation that the Food and Drug Administration (FDA) will possibly convey revised standards of approval regarding vector testing for helper virus contamination. The following section may be added to Appendix D:

"Appendix D-XXXV.
"Dr. Friedrich G. Schuening, Fred Hutchinson Cancer Research Center, Seattle, Washington, can conduct human gene transfer experiments on patients ≥ 18 years of age with breast cancer, Hodgkin's disease, or non-Hodgkin's lymphoma. A total of 10 patients per year will be enrolled in the studies over a period of four years. Patients will undergo autologous bone marrow transplantation with a selected population of Interleukin-3 (IL-3) or granulocyte colony-stimulating factor (G-CSF) stimulated CD34(+) peripheral blood repopulating cells (PBRC) that have been transduced with the gene coding for neomycin resistance (neo^R) using the retroviral vector, LN. Patients will be continuously monitored for neo^R to determine the relative contribution of autologous PBRCs to long-term hematopoietic reconstitution. Demonstration of long-term contribution of autologous PBRC to hematopoiesis will enable the use of PBRC alone for autologous transplants and suggest the use of PBRC as long-term carriers of therapeutically relevant genes."

I accept this recommendation and Appendix D-XXXV of the *NIH Guidelines* will be added accordingly.

D. Addition of Appendix D-XXXVI to the *NIH Guidelines*

In a letter dated July 17, 1992, Dr. Friedrich G. Schuening, Fred Hutchinson Cancer Research Center, Seattle, Washington, submitted a human gene transfer protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of the protocol is *A Trial of G-CSF Stimulated Peripheral Blood Stem Cells for Engraftment in Identical Twins*. This request was published for comment in the *Federal Register* of August 19, 1992 (57 FR 37680).

The protocol was reviewed and recommended for approval during the RAC meeting on September 14-15, 1992, by a vote of 11 in favor, 2 opposed, and 4 abstentions, with the notation that the FDA will possibly convey revised standards of approval

regarding vector testing for helper virus contamination. The following section may be added to Appendix D:

"Appendix D-XXXVI.
"Dr. Friedrich G. Schuening, Fred Hutchinson Cancer Research Center, Seattle, Washington, can conduct human gene transfer experiments on patients ≥ 18 years of age with breast cancer, Hodgkin's disease, or non-Hodgkin's lymphoma. A total of 5 patients per year will be enrolled in the study over a period of four years. Patients will undergo allogeneic bone marrow transplant with granulocyte colony-stimulating factor (G-CSF) stimulated CD34(+) PBRC harvested from an identical twin that have been transduced with neo^R using the retroviral vector, LN. Patients will be continuously monitored for neo^R to determine the relative contribution of G-CSF stimulated allogeneic PBRCs to long-term bone marrow engraftment. Demonstration of long-term contribution of allogeneic PBRC to hematopoiesis will enable the use of PBRC alone for allogeneic transplants and suggest the use of PBRC as long-term carriers of therapeutically relevant genes."

I accept this recommendation and Appendix D-XXXVI of the *NIH Guidelines* will be added accordingly.

E. Addition of Appendix D-XXXVII to the *NIH Guidelines*

In a letter dated June 5, 1992, Dr. Malcolm K. Brenner of St. Jude Children's Research Hospital, Memphis, Tennessee, and Dr. Bonnie J. Mills of Baxter Healthcare Corporation, Santa Ana, California, indicated their intention to submit a human gene transfer protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: *A Phase II Trial of the Baxter Neuroblastoma Bone Marrow Purging System Using Gene Marking to Assess Efficacy*. This request was published for comment in the *Federal Register* of August 19, 1992 (57 FR 37680).

The protocol was reviewed and recommended for approval during the RAC meeting on September 14-15, 1992, by a vote of 19 in favor, 0 opposed, and no abstentions. The following section may be added to Appendix D:

"Appendix D-XXXVII.
"Dr. Malcolm K. Brenner of St. Jude Children's Hospital, Memphis, Tennessee, and Dr. Bonnie J. Mills of Baxter Healthcare Corporation, Santa Ana, California, can conduct a multicenter uncontrolled human gene transfer experiment on 12 patients >21 years of age with Stage D Neuroblastoma

in first or second marrow remission. Autologous bone marrow cells will be separated into two fractions, purged and unpurged. Each fraction will be transduced with the neo^R gene by either LNL6 or G1Na. Patients will be monitored by the polymerase chain reaction (PCR) for the presence of neo^R. The protocol is designed to evaluate the safety and efficacy of the Neuroblastoma Bone Marrow Purging System following high dose chemotherapy."

I accept this recommendation and Appendix D-XXXVII of the *NIH Guidelines* will be added accordingly.

F. Addition of Appendix D-XXXVIII to the NIH Guidelines

In a letter dated July 1, 1992, Drs. Carolyn Keierleber and Ann Progulske-Fox of the University of Florida, Gainesville, Florida, requested permission to conduct experiments which involve the introduction of a gene coding for tetracycline resistance into *Porphyromonas gingivalis* at Biosafety Level 2. This request was published for comment in the *Federal Register* of August 19, 1992 (57 FR 37680).

The protocol was reviewed and recommended for approval during the RAC meeting on September 14-15, 1992, by a vote of 15 in favor, 0 opposed, and 1 abstention. The following section may be added to Appendix D:

"Appendix D-XXXVIII.

"Drs. Carolyn Keierleber and Ann Progulske-Fox of the University of Florida, Gainesville, Florida, can conduct experiments involving the introduction of a gene coding for tetracycline resistance into *Porphyromonas gingivalis* at a physical containment level of Biosafety Level-2 (BL-2)."

I accept this recommendation and Appendix D-XXXVIII of the *NIH Guidelines* will be added accordingly.

G. Addition of Appendix D-XXXIX to the NIH Guidelines

In a letter dated May 10, 1990, Dr. Scott M. Freeman of the University of Rochester School of Medicine, Rochester, New York, indicated his intention to submit a human gene therapy protocol to the Human Gene Therapy Subcommittee (HGTS) and the RAC for formal review and approval. The title of this protocol is: "Gene Transfer for the Treatment of Cancer." This request was published for comment in the *Federal Register* on July 2, 1991 (56 FR 30398).

The protocol was reviewed during the HGTS meeting on July 29-30, 1991. Provisional approval was given with the

stipulation that the PA-1 ovarian cancer cell line be tested for potential pathogens as per FDA guidelines.

Further, it was requested that more preclinical studies on the MFG vector should be performed in order to assure the absence of replication-competent retroviruses. Dr. Freeman submitted the requested documentation to ORDA. The HGTS forwarded the protocol to the RAC for consideration during the October 7-8, 1991, meeting. This request was published for comment in the *Federal Register* on September 3, 1991 (56 FR 43686).

The protocol was reviewed during the RAC meeting on October 7-8, 1991. The RAC passed a motion to defer approval of the protocol by a vote of 19 in favor, 0 opposed, and no abstentions. The RAC stated that the protocol may be considered again when the following criteria have been met:

(i) Improve the animal model so that it has some relevance to the malignancy that is observed in patients;

(ii) Examine the animal model for the tumor specificity of cytotoxic T lymphocytes;

(iii) Demonstrate the efficacy of this proposed treatment by measuring the tumor burden in patients and state whether this will be done by laparoscopy, imaging, or both;

(iv) Refine safety tests; and

(v) Eliminate every reference to the term cancer vaccine in the patient consent form.

In a telephone conversation with Dr. Wivel on January 2, 1992, and a follow-up letter dated January 7, 1992, Dr. Scott M. Freeman indicated his intention to resubmit his revised protocol to the RAC for formal review and approval. The request was published for comment in the *Federal Register* on January 3, 1992 (57 FR 316).

During the meeting on February 10, 1992, the RAC met to review the protocol. There was discussion regarding the safety data, and the use of the term *vaccine*. The investigator agreed to delete all reference to the term *vaccine* since the protocol is designed to evaluate the effect of TK+ on TK- cells, not an immune response. After lengthy discussion, the RAC, by a vote of 10 in favor, 4 opposed and no abstentions, approved the protocol pending receipt of additional safety data that demonstrates transduced, irradiated cells are not producing replication-competent helper viruses or generating rescued TK+ virus.

On December 10, 1992, Dr. Freeman submitted to ORDA the additional safety data relative to the cell line, PA-1-STK. The data was reviewed by the RAC primary reviewer, and it was

determined that it meets the request of the RAC. The following section may be added to Appendix D:

"Appendix D-XXXIX.

"Dr. Scott M. Freeman of Tulane University Medical Center, New Orleans, Louisiana, can conduct experiments on patients with epithelial ovarian carcinoma who have clinical evidence of recurrent, progressive, or residual disease who have no other therapy available to prolong survival. Patients will be injected intraperitoneally with the irradiated PA-1 ovarian carcinoma cell line which has been transduced with the herpes simplex thymidine kinase (HSV-TK) gene. The patients will then receive ganciclovir therapy. Previous data indicates that HSV-TK+ tumor cells exhibit a killing effect on HSV-TK- cells when exposed to ganciclovir therapy. Patients will be evaluated for safety and side effects of this treatment."

I accept this recommendation and Appendix D-XXXIX of the *NIH Guidelines* will be added accordingly.

II. Summary of Actions

A. Addition to the Points to Consider in the Design and Submission of Protocols for the Transfer of Recombinant DNA into the Genome of Human Subjects Regarding Submission Requirements for Human Gene Transfer/Gene Therapy Protocols

The following section is added to the *Points to Consider*:

"Guidelines for the Submission and Review of Human Gene Transfer/Therapy Protocols for Review by the Recombinant DNA Advisory Committee

"I. Investigator-Submitted Material:

"Written proposals should begin with the lay and scientific abstracts, followed by the *Points to Consider* and the material provided in the body of an R01 (sections A-D). When a proposal has been submitted previously, there should be a short section (≤200 words) immediately following the abstracts that summarizes major revisions since the last review. Length limitations are 4-5 pages for the *Points to Consider*, 2 pages each for CVs (Biosketch format) and 20 pages for the body of the proposal (excluding tables, figures, appendices, and manuscripts). Data provided must include a description of the elements in the vector, the source of that information, and the method by which sequence data were compiled, plus 3 diskettes with the vector sequence in ASCII format. Written material from Principal Investigators must be submitted ≥8 weeks before the meeting

at which it will be reviewed; written comments from the primary reviewers ≥ 4 weeks before the RAC meeting; and written responses from the Principal Investigators ≥ 2 weeks before the meeting.

"Oral Presentations at RAC Meetings provide only a brief overview of the proposal; they should concentrate on questions raised by the reviewers before and at the meeting. Oral presentations should be ≤ 20 minutes; ≤ 10 minutes for the overview and ≤ 10 minutes for responses to the reviewers' questions.

"II. Reviewers' Responses:

"Written Reviews should emphasize issues related to gene marking, gene transfer or gene therapy. They should state explicitly whether the *Points to Consider* have been addressed satisfactorily, and should examine the scientific rationale, scientific context (relative to other proposals reviewed by the RAC), whether the preliminary *in vitro* and *in vivo* data were obtained in appropriate models and are sufficient, and whether questions related to safety, efficacy, and social and ethical context have been resolved. Whenever possible, criticisms of Consent Forms should include suggested revisions for the RAC to consider—provided as written alternatives. Reviews should also state whether the proposal is:

"(a) Acceptable as written,

"(b) Acceptable with specific revisions or after satisfactory responses to specific questions raised on review, or

"(c) Unacceptable in its present form.

"Oral Discussion of Reviews at the RAC Meeting. It should be possible to present most reviews orally within ≤ 5 minutes."

B. Addition of Appendix D-XXXIV to the NIH Guidelines

The following section is added to Appendix D:

"Appendix D-XXXIV.

"Dr. Michael T. Lotze, University of Pittsburgh, Pittsburgh, Pennsylvania, can conduct experiments on twenty patients with metastatic, and/or unresectable, locally advanced melanoma, renal cell carcinoma, breast cancer, or colon cancer who have failed standard therapy. Patients will receive multiple subcutaneous injections or autologous tumor cells combined with an autologous fibroblast cell line that has been transduced *in vitro* with the gene coding for Interleukin-4 (IL-4) to augment the *in vivo* antitumor effect. Patients will be monitored for antitumor effect by PCR analysis and multiple biopsy of the injection site."

C. Addition of Appendix D-XXXV to the NIH Guidelines

The following section is added to Appendix D:

"Appendix D-XXXV.

"Dr. Friedrich G. Schuening, Fred Hutchinson Cancer Research Center, Seattle, Washington, can conduct human gene transfer experiments on patients ≥ 18 years of age with breast cancer, Hodgkin's disease, or non-Hodgkin's lymphoma. A total of 10 patients per year will be enrolled in the studies over a period of four years. Patients will undergo autologous bone marrow transplantation with a selected population of Interleukin-3 (IL-3) or granulocyte colony-stimulating factor (G-CSF) stimulated CD34(+) peripheral blood repopulating cells (PBRC) that have been transduced with the gene coding for neomycin resistance (neo^R) using the retroviral vector, LN. Patients will be continuously monitored for neo^R to determine the relative contribution of autologous PBRCs to long-term hematopoietic reconstitution. Demonstration of long-term contribution of autologous PBRC to hematopoiesis will enable the use of PBRC alone for autologous transplants and suggest the use of PBRC as long-term carriers of therapeutically relevant genes."

D. Addition of Appendix D-XXXVI to the NIH Guidelines

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"Dr. Friedrich G. Schuening, Fred Hutchinson Cancer Research Center, Seattle, Washington, can conduct human gene transfer experiments on patients ≥ 18 years of age with breast cancer, Hodgkin's disease, or non-Hodgkin's lymphoma. A total of 5 patients per year will be enrolled in the study over a period of four years. Patients will undergo allogeneic bone marrow transplant with granulocyte colony-stimulating factor (G-CSF) stimulated CD34(+) PBRC harvested from an identical twin that have been transduced with neo^R using the retroviral vector, LN. Patients will be continuously monitored for neo^R to determine the relative contribution of G-CSF stimulated allogeneic PBRCs to long-term bone marrow engraftment. Demonstration of long-term contribution of allogeneic PBRC to hematopoiesis will enable the use of PBRC alone for allogeneic transplants and suggest the use of PBRC as long-term carriers of therapeutically relevant genes."

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"Dr. Malcolm K. Brenner of St. Jude Children's Hospital, Memphis, Tennessee, and Dr. Bonnie J. Mills of Baxter Healthcare Corporation, Santa Ana, California, can conduct a multicenter uncontrolled human gene transfer experiment on 12 patients ≤ 21 years of age with Stage D Neuroblastoma in first or second marrow remission. Autologous bone marrow cells will be separated into two fractions, purged and unpurged. Each fraction will be transduced with the neo^R gene by either LNL6 or G1Na. Patients will be monitored by the polymerase chain reaction (PCR) for the presence of neo^R. The protocol is designed to evaluate the safety and efficacy of the Neuroblastoma Bone Marrow Purging System following high dose chemotherapy."

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G. Addition of Appendix D-XXXIX to the NIH Guidelines

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OMB's "Mandatory Information Requirements for Federal Assistance

Program Announcements" (45 FR 39592) requires a statement concerning the official government programs contained in the *Catalog of Federal Domestic Assistance*. Normally NIH lists in its announcements the number and title of affected individual programs for the guidance of the public. Because the guidance in this notice covers not only virtually every NIH program but also essentially every Federal research program in which DNA recombinant

molecule techniques could be used. It has been determined to be not cost effective or in the public interest to attempt to list these programs. Such a list would likely require several additional pages. In addition, NIH could not be certain that every Federal program would be included as many Federal agencies, as well as private organizations, both national and international, have elected to follow the *NIH Guidelines*. In lieu of the individual

program listing, NIH invites readers to direct questions to the information address above about whether individual programs listed in the *Catalog of Federal Domestic Assistance* are affected.

Effective Date: February 5, 1993.

Bernadine Healy,

Director, National Institutes of Health.

[FR Doc. 93-3820 Filed 2-17-93; 8:45 am]

BILLING CODE 4140-01-M

RECOMBINANT DNA ADVISORY COMMITTEE

Minutes of Meeting

March 1-2, 1993

U.S. DEPARTMENT OF HEALTH
AND HUMAN SERVICES
Public Health Service
National Institutes of Health

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DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

RECOMBINANT DNA ADVISORY COMMITTEE

MINUTES OF MEETING¹

March 1-2, 1993

The Recombinant DNA Advisory Committee (RAC) was convened for its fifty-third meeting at 9:00 a.m. on March 1, 1993, at the National Institutes of Health, Building 31, Conference Room 6, 9000 Rockville Pike, Bethesda, Maryland 20892. Dr. LeRoy B. Walters (Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public. The following were present for all or part of the meeting:

Committee members:

Constance E. Brinckerhoff, Dartmouth Medical School
Nancy L. Buc, Weil, Gotshal, and Manges
Ira H. Carmen, University of Illinois
Gary A. Chase, Johns Hopkins University
Patricia A. DeLeon, University of Delaware
Krishna R. Dronamraju, Foundation for Genetic Research
E. Peter Geiduschek, University of California, San Diego
Mariann Grossman, University of Michigan Medical Center
Robert Haselkorn, University of Chicago
Susan S. Hirano, University of Wisconsin
Donald J. Krogstad, Tulane University School of Medicine
Brigid G. Leventhal, Johns Hopkins Hospital
Abbey S. Meyers, National Organization for Rare Disorders
A. Dusty Miller, Fred Hutchinson Cancer Research Center
Arno G. Motulsky, University of Washington Medical School
Robertson Parkman, Childrens Hospital of Los Angeles
Leonard E. Post, Parke-Davis Pharmaceutical Division
Marian G. Secundy, Howard University College of Medicine
Brian R. Smith, Yale University School of Medicine
Stephen E. Straus, National Institutes of Health
LeRoy B. Walters, Kennedy Institute of Ethics, Georgetown University
Doris T. Zallen, VA Polytechnic Institute & State University

¹The RAC is advisory to the National Institutes of Health (NIH), and its recommendations should not be considered as final or accepted. The Office of Recombinant DNA Activities should be consulted for NIH policy on specific issues.

Executive secretary:

Nelson A. Wivel, National Institutes of Health

A committee roster is attached (Attachment).

Non-voting agency representatives:

Bernard Greifer, Department of Commerce

National Institutes of Health staff:

Leon Baltrucki, NHLBI
Bobbi Bennett, OD
Roscoe Brady, NINDS
Steven Brody, NHLBI
Jan Casadei, NCI
Ken Cowan, NCI
Chin-Shyan Chu, NHLBI
Judy Fradkin, NIDDK
MaryEllen Franko, NCI
Barry Goldspiel, CC
Michael Gottesman, NCI
Roberta Haber, NIDDK
Jeffrey Hoeg, NHLBI
Christine Ireland, OD
Sachiko Kajigaya, NHLBI
Becky Lawson, OD
Charles Link, NCI
Fred Lombardo, NHLBI
Kathryn McKeon, NIDDK
Arthur Neinhuis, NHLBI
Melissa Rosenfeld, NHLBI
Gene Rosenthal, NHLBI
Nava Sarver, NIAID
Toshihiro Soma, NHLBI
Brian Sorrentino, NHLBI
Bob Wersto, NHLBI
Debra Wilson, OD

Others:

Paul Aebersold, Food and Drug Administration
Nicholas Akdemir, Genetic Therapy, Inc.
French Anderson, University of Southern California
Arthur Bank, Columbia University
Jack Barber, Viagene, Inc.
James Barrett, Genetic Therapy, Inc.
Edward Beecham, Genetic Therapy, Inc.
Arie Belldegrun, University of California, Los Angeles
Anton Berns, Somatix Therapy Corporation
Richard Boucher, University of North Carolina, Chapel Hill
Liz Bowie, The Baltimore Sun
G'dali Braverman, Act Up
Malcolm Brenner, St. Jude Childrens Research Hospital
Lou Bucalo, Ingenex, Inc.
Barrie Carter, Targeted Genetics, Inc.
Jan Chappell, Genetic Therapy, Inc.
Ben Cheng, Act Up
Yawen Chiang, Genetic Therapy, Inc.
Shirley Clift, Somatix Corporation
Larry Cohen, Somatix Corporation
Kenneth Culver, University of Iowa
Timothy Darrow, Duke University
Krista Delviks, Hood College
Wanda de Vlaminck, Avigen Corporation
Ross Donehorner, Johns Hopkins University
Jianyun Dong, University of Alabama
Ron Dorazio, Genetix, Inc.
Glen Dronoff, Johns Hopkins University
Anne Driscoll, Novus Group
Michelle Durand, The French Embassy
David Ennist, Genetic Therapy, Inc.
Susan Falen, Genetic Therapy, Inc.
Mitchell Finer, Cell Genesys, Inc.
Stacy Fitzsimmons, Cystic Fibrosis Foundation
Suzanne Forry-Schaudies, Genetic Therapy, Inc.
Jeffrey Fox, ASM News and Biotechnology
Ray Frizzell, University of Alabama
Siqang Fu, MD Anderson Cancer Research Center
Ram Gamgavalli, Viagene, Inc.
Richard Giles, University of Illinois

Steve Goff, Columbia University
Beth Gregory, Johns Hopkins University
Kurt Gunter, Food and Drug Administration
Ingo Haertel, Georgetown University
Karen Hauda, Johns Hopkins University
Charles Hesdorffer, Columbia University
Helen Heslop, St. Jude Childrens Research Hospital
Tom Horiagon, Chimerix Corporation
Zafer Iqbal, University of Illinois
Liz Jaffae, Johns Hopkins University
John Jaugstetter, Genentech, Inc.
Dorothy Jessop, U.S. Department of Agriculture
Larry Johnson, University of North Carolina, Chapel Hill
Douglas Jolly, Viagene, Inc.
John Kavanagh, MD Anderson Cancer Research Center
Rachel King, Genetic Therapy, Inc.
Don Koho, Childrens Hospital of Los Angeles
Toshi Kotani, Genetic Therapy, Inc.
Mike Knowles, University of North Carolina, Chapel Hill
Maryann Krane, Genzyme Corporation
Edward Lanphier, Somatix Corporation
Kyle Legg, Legg Mason
Ronald Leonardi, R & R Registrations
Hy Levitsky, Johns Hopkins University
Fray Marshall, Johns Hopkins University
Michael McCaughan, The Blue Sheet
Alan McClelland, Genetic Therapy, Inc.
Gerard McGarrity, Genetic Therapy, Inc.
Bruce Merchant, Viagene, Inc.
Nanette Mitterder, Genetic Therapy, Inc.
Robert Moen, Genetic Therapy, Inc.
Lisa Morris, Genetic Therapy, Inc.
Steve Mueller, Genetic Therapy, Inc.
Richard Mulligan, Massachusetts Institute of Technology
William Nelson, Johns Hopkins University
Albert Owens, Johns Hopkins University
Drew Pardoll, Johns Hopkins University
Seth Pauker, Food and Drug Administration
Steven Piantadori, Johns Hopkins University
Rick Pickles, University of North Carolina, Chapel Hill
Eckhard Podack, University of Miami
Bob Powell, University of Miami

Paul Recer, The Associated Press
Rex Rhein, Biotechnology News Watch
Bob Riley, Genetic Therapy, Inc.
Joseph Rokovich, Somatix Therapy, Inc.
Ivor Royston, San Diego Cancer Research Center
H. F. Seigler, Duke University
G. Terry Sharrer, Smithsonian Institution
Rachel Sheppard, Genetic Therapy, Inc.
Tomiko Shimada, Ambience Awareness International
Jonathan Simons, Johns Hopkins University
Sharon Smith, Hood College
Robert Sobol, San Diego Cancer Research Center
Eric Sorscher, University of Alabama
Kasi Sridhar, University of Miami
Jim Timmins, Agricetus Corporation
Paul Tolstoshev, Genetic Therapy, Inc.
Bruce Trapnell, Genetic Therapy, Inc.
Carol Trapnell, Food and Drug Administration
John Van Gilder, University of Iowa
Lucy Vulchanova, Hood College
Michael Walsh, Robertson Stephens & Company
John Warner, Viagene, Inc.
Lisa White, The Blue Sheet
Jim Wilson, University of Michigan
Robert Wilmott, Children's Hospital in Cincinnati
Jim Zabora, Johns Hopkins University
Zhaoqing Zhou, University of North Carolina, Chapel Hill

I. CALL TO ORDER

Dr. Walters (Chair) called the meeting to order and stated that notice of this meeting was published in the *Federal Register* as required by the *National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)*. He noted that a quorum was present and outlined the order in which speakers would be recognized. The primary and secondary reviewers will present their comments regarding the protocol, followed by responses from the principal investigators (PIs). The Chair will then recognize other committee members, *ad hoc* consultants, other NIH and Federal employees, the public who have submitted written statements prior to the meeting, followed by the public at large.

Dr. Walters welcomed the following new members of the Recombinant DNA Advisory Committee (RAC): Ms. Abbey Meyers of the National Organization for Rare Disorders, New Fairfield, Connecticut; Dr. Arno Motulsky of the University of Washington Medical School, Seattle, Washington; Dr. Brian Smith of Yale University School of Medicine, New Haven, Connecticut; Dr. Stephen Straus of the NIH, Bethesda, Maryland; and Ms. Mariann Grossman of the University of Pennsylvania, Philadelphia, Pennsylvania.

II. MINUTES OF THE DECEMBER 3-4, 1992, AND JANUARY 14, 1993, MEETINGS

Dr. Walters called on Dr. DeLeon to review the minutes of the December 3-4, 1992, RAC meeting. Dr. DeLeon stated that the minutes were an accurate reflection of the committee's deliberations. Minor corrections were submitted by Dr. Geiduschek and Ms. Meyers. The RAC unanimously approved a motion made by Dr. DeLeon and seconded by Dr. Krogstad to accept the December 3-4, 1992, RAC minutes with the inclusion of minor grammatical changes by a vote of 19 in favor, 0 opposed, and no abstentions.

Dr. Walters called on Dr. Chase to review the minutes of the January 14, 1993, RAC meeting. Dr. Chase stated that the minutes were an accurate reflection of the committee's deliberations. Minor corrections were submitted by Drs. Chase, Geiduschek, Zallen, and Carmen. The RAC unanimously approved a motion, made by Dr. Dronamraju and seconded by Dr. Zallen to accept the January 14, 1993, RAC minutes with the inclusion of minor grammatical changes by a vote of 19 in favor, 0 opposed, and no abstentions.

III. ADDITION TO APPENDIX D OF THE NIH GUIDELINES REGARDING A HUMAN GENE THERAPY PROTOCOL ENTITLED: *GENE THERAPY FOR THE TREATMENT OF MALIGNANT BRAIN TUMORS WITH IN VIVO TUMOR TRANSDUCTION WITH THE HERPES SIMPLEX-THYMIDINE KINASE GENE/GANCICLOVIR SYSTEM*/DRS. CULVER AND VAN GILDER

Review--Dr. Hirano

Dr. Walters called on Dr. Hirano to present her primary review of the protocol submitted by Dr. Kenneth W. Culver of Iowa Methodist Medical Center, Des Moines, Iowa, and John C. Van Gilder of the University of Iowa, Iowa City, Iowa.

Dr. Hirano provided a brief overview of the protocol. This study is similar another protocol that was submitted by Dr. Edward Oldfield, and approved by the RAC at its June 1992 meeting. A retrovirus vector will be used to integrate the gene coding for herpes simplex-thymidine kinase (HS-tk) into the brain tumor cells of patients. In turn, the HS-tk gene renders these cells sensitive to the antiviral drug ganciclovir (GCV). HS-tk will be delivered to the patient's brain tumor cells by direct injection of the murine vector producing cells (VPC), PA317. Animal model studies performed in mice, rats, and nonhuman primates indicate that no significant toxicity is observed from the direct injection of VPC into the brain.

This study is designed to assess the safety and efficacy of the repeated administration of VPC followed by GCV treatment in patients with malignant brain tumors. In addition, patients will be monitored for systemic immunity resulting from the repeated injection of murine cells. A total of 40 patients will be entered into this study: 20 patients at the University of Iowa and 20 patients at Iowa Methodist Medical Center. Patient eligibility is limited to those individuals who have surgically accessible brain tumors.

The investigators propose to administer 3 injections of the VPC, each dose containing approximately 1×10^9 cells in a maximum volume of 10 milliliters (ml). Dr. Hirano asked the investigators to explain their reason for keeping the number of cells constant for all patients rather than proposing a dose escalation. On day 1, patients will undergo surgical debulking of their tumor and receive the initial injection of VPC. The second injection of cells will be administered on day 7 followed by GCV treatment on day 14; the original schema proposed an entire treatment cycle of 2 weeks. However, the investigators now propose a revised treatment schema which includes a third injection of VPC and subsequent GCV administration.

Dr. Hirano inquired whether the revised treatment schema is absolute for all patients or whether there is some degree of flexibility in the administration schedule. What is the possibility that any or all of the patients will require a third injection of VPC and subsequent GCV administration? Dr. Culver responded that the treatment schema for the first two injections is absolute; however, there will be flexibility in the administration of the third injection. If there is evidence of tumor recurrence, then the patient will receive the additional treatment. Dr. Hirano said that the schema described by Dr. Culver is not clearly outlined in the protocol.

Dr. Hirano said that there is no evidence that the third treatment is necessary. How long does GCV persist in the body? Is there data to demonstrate that the proposed concentration of GCV will not kill the VPC before the HS-tk gene has been integrated into the patient's brain tumor cells? Regarding the safety of the VPC, what is the minimum concentration of VPC per ml that will be produced per production lot? Will all of the proposed safety studies be performed on only a portion of each production lot? Are the assays employed for the detection of replication-competent retroviruses (RCR) sensitive enough to detect less than one RCR particle per volume, i.e., 200 ml? Are the VPC cryopreserved? If not, are these cells stable after several weeks in culture? What is the timetable from the production of the VPC to the time that they are administered to the patient? She noted that the investigators did not include the possibility of a third treatment in the informed consent document. She requested that the investigators provide an update regarding Dr. Oldfield's approved glioblastoma protocol, on which Dr. Culver is a co-investigator.

Review--Dr. Geiduschek

Dr. Geiduschek stated that the investigators had provided adequate responses to his concerns regarding the preclinical summary and the informed consent document; however, their response regarding the safety of the PA317 packaging cell line was inadequate. Dr. Culver stated in his written response that no breakout of RCR has occurred with PA317, and that there is no alternative packaging cell line that can be used for this protocol. Dr. Geiduschek said that although breakout of RCR in PA317 occurs at a relatively low frequency, it is possible. In addition, there are alternative packaging cells lines that could be used for this protocol.

Dr. Geiduschek suggested that the problems associated with RCR breakout in PA317 might be minimized by implementing the following procedures: (1) limit the period of time that the PA317 cells are expanded in culture, (2) cryopreserve the PA317 cells while a portion of these cells is grown in culture for a defined period of time to monitor for RCR breakout, or (3) use a new generation of packaging cells, i.e., Ψ -CRIP producer cells.

Other Comments

Dr. Miller stated that new packaging cell lines are being developed with split genomes in which the *gag* and *env* portions are separated. A canine packaging cell line is currently being developed that would eliminate the problems associated with murine helper virus production. However, the developer of this canine cell line recently reported an incident of helper virus production. Therefore, using a split genome packaging cell line does not necessarily guarantee safety. Since there is no method available to determine whether helper virus will be produced in the patient's brain, the RAC must determine that the

seriousness of the disease justifies this treatment; and that there is a reasonable expectation that this procedure will be therapeutic. Dr. Haselkorn inquired whether the risk of helper virus production could be quantitated. Dr. Miller said that although the probability of detecting helper virus in a production lot could be determined, there are mitigating factors in the human; such as complement which inactivate these viruses.

Dr. Zallen noted that there was a discrepancy between the Iowa Methodist Medical Center and the University of Iowa Informed Consent documents regarding patients' financial obligations. The investigators should address the issue of patient liability in their response.

Dr. Post asked the investigators to respond to the following questions: (1) What is an Ommaya reservoir and what is the history of its use? and (2) What is the status of the glioblastoma protocol which has already been approved by the RAC? Has an immune response to the NIH3T3 cells been observed?

Presentation--Dr. Culver

Dr. Walters called on Dr. Culver to respond to the questions and comments of the RAC members. Dr. Culver explained that one of the main principles of successful tumor therapy is to treat tumor cells that are entering into the proliferative phase. Glioma cells demonstrate a very heterogeneous rate of division; therefore, this therapy will be administered over a period of 60 days in order to optimize gene transfer into the greatest number of dividing tumor cells. Patients who have evidence of recurrent tumor following the initial therapy can be retreated through the Ommaya reservoir if that recurrence is on the margin of the resection.

Dr. Culver said that the half-life of GCV is approximately 12 hours in humans. Data derived from rat and monkey animal models indicates that the producer cells survive for approximately 2 weeks. If the PA317 cells remain viable in humans for this period of time, the proposed treatment schema will allow for maximum gene transfer and provide the greatest opportunity for the bystander effect to occur.

Dr. Culver stated that the description of the third injection of VPC into the Ommaya reservoir had been inadvertently omitted from the informed consent document; the third injection will be included in a revised version. He said that Dr. Gerard McGarrity of Genetic Therapy, Inc. (GTI), the vector supplier, would respond to the committees questions regarding safety testing of the VPC.

Dr. Culver provided a brief update on the Oldfield glioblastoma protocol. To date, 5 patients with surgically inaccessible brain tumors have received a single injection of VPC into various locations at the site of their tumor: 1 patient is currently receiving GCV

therapy, and 4 patients have completed treatment. No instances of toxicity have been observed in response to either VPC administration of GCV therapy. Dr. Culver noted that initial concerns about the possibility of thrombocytopenia and neutropenia in patients recovering from chemotherapy have not been observed. Efficacy data is unavailable at this time.

Dr. Hirano inquired whether the VPC used in Dr. Oldfield's protocol are ever cryopreserved prior to administration to the patient. Dr. Culver responded that the VPC are cryopreserved and thawed immediately before they are to be administered to the patient. A minimal amount of time is allowed to count the cells and examine them for microscopic contamination. Animal studies demonstrate that cryopreserved cells are as effective as cultured cells in eliciting an anti-tumoral response.

With regard to the issue of financial responsibility, GTI is sponsoring this protocol; therefore, patients will not be responsible for any costs associated with the research aspects of this protocol. If surgery is already indicated, as it most likely will, then the costs associated with the surgical procedures will be billed to third party payers. There are differences between the two institutions regarding compensation for research related injury. The Iowa Methodist Medical Center will provide care and compensation for any acute injury related to the gene therapy procedure; there is no mechanism for long-term care. Dr. Culver introduced Dr. Van Gilder to explain the compensation policy of the University of Iowa and to explain the use and history of the Ommaya reservoir.

Presentation--Dr. Van Gilder

Dr. Van Gilder stated that the University of Iowa would cover all costs associated with the research aspects of the protocol, and third party carriers will be billed for costs associated with routine surgical procedures.

Dr. Van Gilder explained that an Ommaya reservoir is a small tubing, approximately 2 millimeters in external diameter, that is placed into the center of the surgical resection. This tubing leads to a cap which is implanted directly under the skin of the skull, allowing for the direct injection of the cells through the skin and into the reservoir. Dr. Miller asked if virus particles can pass through this reservoir? Dr. Culver answered that experiments have been performed demonstrating that high concentrations of virus particles can be passed through the reservoir with no observed decrease in viability. In addition, VPC have been passed through the reservoir and shown to exhibit the same anti-tumor effect as control cells. Dr. Van Gilder explained that Ommaya reservoirs have been used as standard treatment for the administration of chemotherapeutic agents into the cerebral spinal fluid and ventricular system of cancer patients for many years.

Dr. Leventhal asked for a clearer definition of the endpoints for the proposed study.

How will efficacy be assessed? How did the investigators arrive at the proposed accrual of 20 patients? Dr. Van Gilder responded that patients will be evaluated on the basis of their Karnofsky score and by magnetic resonance imaging (MRI) and computerized tomography (CT) scans. Dr. Leventhal said that these procedures will indicate whether the tumor is growing; however, nothing will be known about the efficacy of the treatment. If treatment failure will be indicated by growth of the tumor, how will you know if the treatment has been successful? Is patient eligibility limited to those patients who have measurable tumors on their post-operative CT scan? Dr. Van Gilder said that measurable tumor post surgery would be included as an eligibility requirement.

Dr. Van Gilder explained that it is difficult to distinguish between post-operative and therapeutic effects; therefore, sequential scanning is important. Although responses to treatment may vary, there is a consistency that is observed in response to surgery. Immediately following surgery, it is difficult to distinguish between residual tumor and edema. By the time a patient has completed their chemotherapy regimen, approximately 6 weeks post surgery, the edema has usually disappeared. Changes are generally observed in the enhanced volumes of CT scans at approximately 2 months post surgery. Sequential scanning is essential in evaluating the efficacy of the treatment. Dr. Leventhal asked whether it is possible that patients will be rejected from this study if they have no demonstrable tumor on their post-operative CT scan. Dr. Van Gilder explained that this rejection would be an extremely rare occurrence because malignant gliomas cannot be completely resected.

Dr. Leventhal asked how the residual tumor would be measured. Dr. Van Gilder explained that residual tumor is determined based on the blood brain barrier, i.e., the enhanced volume on the CT scan. Dr. Leventhal stated that the eligibility requirements should be expanded in the informed consent document to include only those patients who have measurable tumor as demonstrated by an immediate post-operative imaging procedure.

Discussion

Dr. Krogstad said that it is unclear how the investigators will determine if the patient's positive or negative response is a direct result of the gene therapy portion of the protocol. Dr. Leventhal agreed with Dr. Krogstad's conclusion and stated that the protocol should include a statistical section which outlines how the objectives of the protocol correlate with the number of patients proposed, i.e., 40 patients. Dr. Culver said that data derived from 14 patients should provide sufficient statistical information to distinguish between false positive and negative responses. Dr. Leventhal explained that 14 patients will statistically prove if there is a 95% chance that 20% of the patients may respond to the treatment. The maximum level of response has to be defined. If this therapy is toxic, then a 20% response rate may be unacceptable. If the first 14 patients

demonstrate no response, will the protocol be terminated? If a positive response is observed in 1 of the first 14 patients, will the remainder of the 40 patients be treated? Dr. Culver agreed to include an expanded section regarding the definition of endpoints as suggested by Dr. Leventhal.

Dr. Chase inquired about the standard of statistical analysis for human gene transfer protocols that are approved by the RAC. Dr. Leventhal stated that investigators should be required to include a section that outlines the objective of the protocol and to describe how "X" number of patients will statistically determine that the objective has been achieved. The investigator is responsible for defining that objective, i.e., toxicity, efficacy, etc.

Dr. Haselkorn asked if the Ommaya reservoir could be used for sampling as well as for the administration of the VPC. Are there biochemical markers which can be used to determine the efficacy of the treatment using reservoir sampling? He asked Dr. Culver to respond to Dr. Post's question regarding immune status of patients that have been treated on the Oldfield protocol. Dr. Culver answered that Ommaya reservoirs are not designed for sampling purposes. The tubing is thin and flexible, aspiration would be difficult.

Dr. Culver responded to Dr. Post's question about the immune status of patients in the ongoing glioblastoma protocol. Cell and serum samples have been cryopreserved for all patients who have received VPC and ganciclovir therapy. These samples will be evaluated in the future for immune responses to PA317. Data indicates that patients receiving 32 milligrams per day of dexamethasone are completely anergic based on immunologic analyses. Dr. Post inquired about the number of cells that patients have received to date on the Oldfield protocol. Dr. Culver answered that 5 patients have received one of the following numbers of VPC: 1.7×10^8 , 5×10^8 , or 1×10^9 cells. There has been no evidence of toxicity in response to any of these doses of VPC. Dr. Walters asked if any unexpected effects had been observed in the 5 patients treated to date. Dr. Culver responded that no unexpected effects have been observed as a result of the implantation of these VPC in patients.

Dr. Smith reiterated that Phase II studies such as this one should include a clearer definition of the criteria for responsiveness. The investigators need to clarify the injection schedule of the VPC. If all patients receive 1 injection of VPC, what are the criteria for more than 1 injection? Dr. Culver said that patients must have demonstrated an initial response. If progressive tumor is observed soon after the first injection, e.g., less than 3 weeks, the patient will not be eligible for further injections. However, if tumor recurrence occurs several months following the initial treatment, another injection of VPC would be administered.

Dr. Hirano inquired about the production and testing procedures for the VPC. Dr. McGarrity explained that there is a master cell bank of the VPC that contains several hundred cryopreserved ampules. Working cell banks are developed from the cryopreserved ampules. A working cell bank contains several hundred ampules. The final production lot of cells is taken from the working cell bank. Extensive quality control testing is performed at every step of this process. The VPC used in the proposed study will probably require two separate production runs. Since the cells are derived from the same working cell bank and the quality control standards are identical, there should be no differences between production runs. Dr. McGarrity explained that Genetic Therapy, Inc., is in the process of expanding the size of its production runs; therefore, alleviating any concerns associated with multiple processes.

Dr. McGarrity addressed the issue of RCR testing. Depending on the production lot, between 2.5 and 5% of each lot is removed for testing purposes. Supernatants are assayed by S⁺L⁻ and NIH3T3 amplification on *Mus dunni* cells. A portion of the VPC are cultured for 3 weeks to ensure that no breakout of RCR occurs.

Committee Motion

A motion was made by Dr. Hirano and seconded by Ms. Meyers to approve the protocol with the following stipulations: (1) Patient eligibility will be limited to those patients with measurable residual tumor on immediate post-operative imaging studies, i.e., CT or MRI scans. (2) Patient enrollment will be limited to 15 patients divided between the Iowa Methodist Medical Center and the University of Iowa. If a positive response is observed in any of the first 15 patients, the investigators may submit a request to treat an additional 15 patients.

Dr. Geiduschek asked for clarification regarding the number of VPC that were removed for extended culture. With the recently approved compassionate use exemption for a human gene transfer protocol, the RAC will have to scrutinize the underlying safety issues more than they have in the past. Dr. McGarrity stated that approximately 2.5% of each production lot undergoes extended culture for 3 weeks in order to monitor potential RCR breakout. Dr. Post inquired as to how this percentage of the production lot compares to a patient dosage. Dr. McGarrity responded that 2.5% of a production lot represents approximately 4 to 5 patient doses. Each production lot contains between 1×10^{10} and 1×10^{11} VPC.

Dr. Miller asked why the RAC was including the stipulation about demonstration of residual tumor if this disease is uniformly fatal. Dr. Leventhal responded that the stipulation would probably not exclude very many patients because it is almost impossible to remove all of the patient's tumor by surgery. If the investigators proposes to establish efficacy based on stable disease for "X" period of time, then they must be

able to define the "X". Stable disease for a period of time is not necessarily evidence of a response; however, stable disease could be a criteria for approving additional injections. There should be a minimum period of disease stability after the third injection before proceeding to a fourth injection.

Dr. Straus inquired about the practicality of determining residual tumor on a post-operative scan. How is residual tumor distinguished from edema? Dr. Van Gilder explained that it is often difficult to determine residual tumor on a post-operative scan; however, sequential scans are beneficial. Dr. Straus agreed with Dr. Miller that demonstration of residual tumor on a post-operative scan may be a moot issue in this uniformly fatal disease. Dr. Leventhal asserted that there are very well defined criteria for distinguishing between residual tumor and edema. However, performing a scan immediately following surgery is critical. Dr. Leventhal stated that if the investigators employ the criteria established by the Brain Tumor Study Group for evaluating residual tumor versus edema, the stipulation would be satisfied.

Dr. Krogstad said that he would be satisfied with the approval to treat 2 or 3 patients; however, the end points of this study and how efficacy will be defined should be addressed before the approval is granted to treat additional patients. Perhaps the approval of additional patients could be handled as a minor modification.

Ms. Meyers asked Dr. Culver how he would respond to any patients who requested to receive this therapy on a compassionate use basis. Dr. Culver said that all patients must meet the eligibility requirements of the protocol. If patients do not meet these requirements or the study is closed, they will be referred to other research protocols in the U.S.

Dr. Leventhal suggested a third stipulation for approval as follows: (3) After the initial 3 injections of the VPC and GCV therapy, patients only will be eligible for additional treatments if they have demonstrated stable disease for a minimum of 6 months. Dr. Hirano and Ms. Meyers accepted Dr. Leventhal's additional stipulation as part of the motion for approval of the protocol. The protocol was approved with stipulations by a vote of 19 in favor, 0 opposed, and no abstentions.

IV. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES REGARDING A HUMAN GENE THERAPY PROTOCOL ENTITLED: PHASE I STUDY OF GENE THERAPY FOR BREAST CANCER/DR. BANK*

Review--Dr. Parkman

Dr. Walters called on Dr. Parkman to present his primary review of the protocol submitted by Dr. Arthur Bank of Columbia University, New York, New York.

Dr. Parkman provided background information about the multi-drug resistance (MDR) gene. The MDR gene encodes a protein that can export chemotherapeutic agents out of cells. High concentrations of MDR are present in hematopoietic stem cells; however, the level of MDR expression is greatly reduced following differentiation. Since the protection provided by MDR is diminished in differentiated cells, chemotherapy usually produces a significant decrease in a patient's white blood cell and platelet counts.

The objective of this protocol is to insert the gene coding for MDR, which is linked to a promoter that maintains gene expression, into hematopoietic stem cells. The patient's cells would be relatively resistant to the effects of certain anti-tumor drugs which allow them to receive increased doses of these chemotherapeutic agents. Increasing the doses of these agents should increase the likelihood that the patient's tumor cells will be ablated.

The investigators propose to use a Harvey murine leukemia virus vector; they have submitted preclinical data based on a murine model that demonstrates their ability to transduce hematopoietic stem cells with MDR and to confer a protective effect against several chemotherapeutic agents, particularly Taxol.

Patient eligibility is limited to women with Stage IV breast cancer who are undergoing autologous bone marrow (ABM) transplantation. Approximately one-third of the patient's bone marrow will be enriched for CD34(+) cells, transduced with the MDR retrovirus vector, and cryopreserved. At the time of transplantation, the patient will receive a mixture of transduced and untransduced ABM cells. The untransduced portion of the patient's ABM cells is sufficient for hematopoietic engraftment. Following transplantation, the patient will be monitored by polymerase chain reaction (PCR) for the MDR gene. The patient's cells will be examined for colony growth, i.e., resistance to the effects of various chemotherapeutic agents.

Patients who demonstrate persistent or progressive disease at day 50 will receive Taxol administration. Following Taxol, patients will be monitored for any increase in the proportion of MDR-expressing cells resulting from the ablation of cells that do not express the MDR gene.

Dr. Parkman said that there are still several remaining questions about this protocol. A hard copy of the complete vector sequence was not made available to all of the members of the committee although Dr. Miller has screened the sequence by computer analysis and has assured the committee that the vector is acceptable. The investigators have not provided any long-term culture or animal data demonstrating that the MDR gene has been incorporated in relatively immature hematopoietic progenitors. The submitted data is based on days 12 and 14 short-term cultures. The investigators have not demonstrated that they can transduce the gene into immature hematopoietic cells and that the progeny

of these cells express the MDR gene. In addition, the investigators have not stated the length of time that they expect to observe gene expression in these patients. How long will patients have to be followed after ABM transplantation to demonstrate the presence of resistant colonies and PCR positive cells, which is indicative of transduced committed progenitors?

The administration of Taxol is another aspect of this protocol that should be considered by the RAC. Taxol is not routinely administered following ABM for breast cancer at Columbia University. Since the administration of Taxol is a procedure that is not independent of the gene transfer procedure, then the committee has a responsibility to review all aspects of the protocol. Dr. Parkman stated that his written review asked if patients tolerated 175 milligrams per meter squared (mg/m^2) of Taxol 50 days following ABM transplantation. The investigators responded that one patient had received this dose of Taxol, 175 mg/m^2 ; however, the drug was administered on day 90 instead of day 50. He stated that data obtained from 1 patient treated on day 90 following ABM does not justify the treatment of many patients on day 50 following ABM.

Dr. Parkman summarized the questions that the investigators should respond to during their oral presentation: (1) Is there preclinical data demonstrating gene transduction into immature hematopoietic progenitors as opposed to committed progenitors? (2) What are the endpoints of the study? and (3) Is there any additional clinical data regarding the safety of Taxol administration at the dose proposed for this study?

Review--Dr. Krogstad

Dr. Krogstad stated that the investigators had responded adequately to his concern about the use of the term MDR-1 for both the genomic DNA message and the gene product. It is unclear how gene expression resulting from the transduced gene will be distinguished from the natural expression of MDR from genomic DNA. Since 1 of the objectives of this study is to select for MDR-1 producing cells or cells that exhibit greater levels of p-glycoprotein, the investigators should provide further clarification on this issue.

One of the specific objectives of this protocol is to assess the toxicity of the treatment. Due to the large number of variables within this protocol, i.e., ABM transplantation and Taxol administration, it is likely that any toxicity resulting from the gene transfer portion of the protocol will be missed.

Dr. Krogstad said that there is concern about the possibility that malignant cells in the patient's bone marrow might be inadvertently transduced with the MDR gene in addition to the normal cells. Published data in the scientific literature indicates that between 30 and 50% of breast cancer patients have bone marrow involvement; however, the extent

of involvement is unclear. How effectively will tumor cells be removed by the CD34(+) selection process? If tumor cells fail to be removed by the selection procedures, will they survive the *in vitro* culture conditions and inadvertently become transduced? These questions must be satisfactorily answered before patients can be allowed to participate in this protocol.

Other Comments

Dr. Brinckerhoff agreed with Dr. Krogstad's concerns about the possible transduction of tumor cells which would render them resistant to chemotherapy. There are no techniques available at the present time that can detect breast cancer cells that may contaminate the bone marrow.

Ms. Meyers stated that she has several concerns about the informed consent document. All references that gene therapy or gene transfer is safer should be deleted. Gene therapy is still in its very early stages, and all possible outcomes are not yet known. The statement, "Taxol is a new drug which has been shown to be effective in the treatment of breast cancer" should be deleted. Taxol has been approved for the treatment of ovarian cancer, but it is still considered experimental in the treatment of breast cancer. Patients should not be responsible for the experimental costs either personally or through their insurance. This procedure is very experimental, not only the gene therapy portion, but also the bone marrow transplantation procedure. The informed consent document should include a statement recommending that the patient should not be pregnant or planning to become pregnant. A statement should be included about a request for autopsy. The investigators should explain how they would respond to patients who might request this treatment on a compassionate use basis.

Dr. Parkman said that he had requested that the informed consent document be revised in his written comments to the investigators and was disappointed that the revised document was not submitted. With regard to the issue of contaminating breast cancer cells in the bone marrow, this issue has been previously discussed during the review of Dr. Joyce O'Shaughnessy's protocol at the December 1992 RAC meeting. During the December 1992 meeting, Dr. Cynthia Dunbar stated that the majority of breast cancer cells are already MDR positive, and that data from other laboratories has demonstrated that there is a significant reduction in the number of breast cancer cells after CD34(+) selection. The discussion should not focus on concerns of transducing non-MDR expressing tumor cells, but only on the possibility of over-expression of the gene. The CD34(+) selection procedure reduces the total number of cells that are to be transduced, therefore, reducing the relative proportion of contaminating breast cancer cells. Dr. Brian Sorrentino, a co-investigator on Dr. O'Shaughnessy's protocol agreed with the comments made by Dr. Parkman. Dr. Sorrentino noted that one of the differences between the two protocols is that Dr. O'Shaughnessy's protocol included a

salvage therapy with 5-fluorouracil (5-FU) and other chemotherapeutic agents, which are not MDR pump drugs; therefore, their effectiveness would not be affected by MDR gene transduction.

Dr. Carmen explained that he had been assigned as a primary reviewer of Dr. Bank's protocol before it was withdrawn for review from the September 1992 RAC meeting. How does the current protocol differ from the previously submitted protocol other than the fact that ovarian cancer is no longer the targeted disease? What is the status of the murine immunodeficiency model? Dr. Carmen then submitted suggested language for inclusion in the informed consent document.

Dr. Post noted that the proposed vector includes 6 additional kilobases which are upstream from the MDR cDNA. What are these additional sequences? Does it make *gag*? Are any additional retrovirus proteins made? Does this MDR vector have the same point mutation and frame shift that Dr. O'Shaugnessy's vector had? What is the status of characterizing the performance of this vector in the proposed packaging cell line? What is the status of Institutional Review Board (IRB) approval of this protocol? The RAC received a document which granted approval in principle; what are the specifics of approval?

Dr. Leventhal stated that all investigators submitting human gene transfer protocols to the RAC should grow and/or purify the target population of human cells and transduce these cells with the same vector proposed for the study. There is no data demonstrating that purified human CD34(+) cells have been transduced with the proposed vector.

Since the target cell population exhibits MDR expression, which decreases as the cells mature, the investigators must demonstrate that the transduced CD34(+) cells express higher levels of MDR than the cells produce spontaneously. What is the effect of transduction?

Dr. Geiduschek stated his concern about the quantitative expression of MDR in the transduced population. The investigators have not submitted data to address the critical question, namely, a comparison between Taxol-selected transduced and untransduced cells. Does Taxol selection cause an increase, decrease, or sustained expression of MDR? The section of the protocol that describes the CD34(+) selection procedure should be clarified. The investigators propose that 30-50% of the total number of bone marrow cells harvested will be removed for CD34(+) selection, depending on the total cell harvest yield. What factors will influence this percentage? The following statement needs to be clarified by the investigator: "We will evaluate the safety of gene transfer by looking for the presence of intact retrovirus in the bone marrow; this is the major risk." The presence of intact retrovirus would be for only a brief duration. Are there other parameters that will be used to monitor the safety of this procedure in patients?

Dr. Miller stated that he has screened the proposed vector. The oncogenes have been removed, and the MDR cDNA extends through approximately 50% of the vector. No other additional open reading frames were identified. The investigators should describe the additional sequences that are at the tail of the vector. Dr. Post inquired about the sequences that are upstream from the MDR cDNA. Dr. Miller said that the upstream sequences are a derivative of a leukemia virus and contain the packaging signal. Dr. Post asked if there was any *gag* upstream from the cDNA. Dr. Miller responded that no *gag* sequences are present. The vector is approximately 9,000 base pairs (bp) from the 5' long terminal repeat (LTR) to the 3' LTR. The MDR cDNA is approximately 4,000 bp.

Dr. Miller explained that the investigators provided data derived from only 1 experiment demonstrating their ability to transduce long-term reconstituting stem cells in mice. This experiment was performed by co-cultivation of the marrow cells with the virus producing cells. However, the human study will use virus supernatant for transfection as opposed to co-cultivation with producer cells. Virus supernatant has been demonstrated to be much less efficient than co-cultivation for transferring genes to bone marrow cells. The virus titer projected for this vector is 10^4 colony forming units (CFU) per ml. This concentration is much less than other investigators have reported as required for successful transduction of bone marrow cells. The *in vivo* transduction data is insufficient to support the proposed study. Dr. Parkman said that the investigators should perform additional long-term *in vitro* human experiments with the proposed vector. Dr. Miller asked the investigators if there is a large animal model that would support this study.

Presentation--Dr. Bank

Dr. Bank introduced his collaborators: Drs. Steve Goff and Charles Hesdorffer of Columbia University, New York, New York, and Dr. Michael Gottesman of NIH, Bethesda, Maryland. Dr. Bank explained that he had reviewed the comments made by the RAC in response to Dr. O'Shaugnessy's protocol, and that this protocol addresses all of the RAC members recommendations. Dr. Bank said that he has PCR data demonstrating that human CD34(+) cells can be transduced with the proposed vector.

In response to Dr. Geiduschek's concerns about MDR expression, Dr. Bank explained that gene expression will be monitored by fluorescence activated cell sorter (FACS) analysis using the 17F9 antibody. When unselected bone marrow cells are transduced with the MDR vector, 10-20% of the cells demonstrate a 5-10-fold increase in MDR expression. One CFU-granulocyte/erythrocyte/megakaryocyte/monocyte (GEMM) resistant colony assay was performed using CD34(+) transduced cells in which 2 of 7 resistant colonies grew. Dr. Bank stated that he is currently in the process of defining the endpoints of this study to determine efficacy.

In response to Dr. Carmen's question about the status of the immunodeficient mouse model, Dr. Bank explained that CD34(+) cells have been transferred to severe combined immunodeficient (SCID) mice; however, this procedure is difficult and the results are inconsistent.

With regard to Taxol administration, Dr. Bank explained that numerous breast cancer patients have received Taxol administration at Columbia University; however, they have not received bone marrow transplants. Dr. Parkman stated that the addition of ABM presents a very different biological setting than Taxol administration alone. Data must be submitted from patients that have received both Taxol and ABM transplantation. This data does not necessarily have to be derived from breast cancer patients. Data from 3 patients does not provide sufficient justification for this protocol.

Other Comments

Dr. Parkman explained that in order to draw a conclusion about the protective effect of the proposed therapy, there must be criteria established for defining a response to Taxol administration alone. Taxol response must be considered in addition to gene transduction and expression. Dr. Hesdorffer responded that patients will continue to be accrued onto Taxol protocols who have had ABM transplantation without transduction. Of the 3 patients who received ABM transplantation and Taxol administration, 1 ovarian cancer and 1 breast cancer patient demonstrated a response. Data is still being accrued on the third patient. Dr. Parkman explained that in order for the RAC to approve this protocol, the risk to the patient must be assessable. Since the response to Taxol administration and untransduced ABM cells alone is unknown, the effect of the inserted MDR gene cannot be evaluated.

Dr. Leventhal stated that the investigators are obligated to define a stopping rule based on their clinical experience if adverse effects occur, i.e., aplasia, resulting from this treatment. Dr. Bank responded that they have defined toxicity criteria for giving patients recurrent doses of Taxol after ABM transplantation, and that Taxol will not be administered to patients until their white counts have reached 300-500.

Dr. Bank responded to Dr. Krogstad's question about distinguishing between gene expression resulting from the transduced gene and natural expression of MDR from genomic DNA. The inserted MDR gene can be distinguished from natural MDR by the following: (1) there is a single mutation that is distinguishable by reverse PCR, (2) glycine is substituted for a valine at position 185, and (3) there are different sequences in the 5' end.

With regard to the assessment of toxicity, Dr. Bank responded that if any patient develops a lymphoma or other type of tumor, the investigators will look for functional

mutagenesis. In addition, vector producing cells will be assayed for replication competent retrovirus. This treatment should not present any added risk to the patient, i.e., administration of MDR-resistant breast cancer cells. In order to impose increased risk to the patient, transduced tumor cells would have to demonstrate biologic virulence that would cause them to repopulate the marrow. Otherwise, there is very little consequence; because it is rare that the bone marrow is the site of tumor recurrence.

Ms. Meyers inquired about the financial responsibility for the costs associated with the proposed study. Dr. Hesdorffer stated that arrangements will be made with third-party carriers to cover the costs associated with the ABM transplantation procedures. Any costs specifically related to the transduction of the cells will not be covered by the institution. Additional procedures such as blood tests, bone marrow harvest, and any pre- and post-transplantation procedures will be billed to third-party carriers.

Ms. Meyers asked who would be responsible for the costs associated with adverse side effects should any arise as a result of the gene transfer procedure. Dr. Bank answered that the patient's third-party carrier would be billed for such costs. Dr. Hesdorffer stated that if toxic side-effects occur as a result of the Taxol administration, a third-party carrier would be charged for costs associated with treatment; however, adverse effects relating to the gene transduction procedure would be covered by Columbia University. Ms. Meyers inquired if the institute would cover any injury not related to Taxol administration or bone marrow transplantation. Dr. Bank agreed that the institution would cover these injuries.

In response to the issue of whether the investigators would seek NIH approval for compassionate use of this protocol for patients who do not meet the eligibility requirements, Dr. Bank said that Columbia University would probably not pursue such a request, because there are numerous breast cancer research protocols to which patients could be referred. He noted that full IRB approval has been obtained and is available.

Dr. Post asked if the investigators have data using the exact packaging cell line and vector that is being proposed of this study. Dr. Bank responded that the only data available with the proposed vector are reverse transcriptase (RT) assays on supernatant from the transduced bone marrow cells. Dr. Bank stated that S⁺ L⁻ assays and co-cultivation with *Mus dunni* cells have not been performed. The proposed packaging cell line has been used with other vectors in over 300 laboratories throughout the world without any incidence of RCR breakout.

Dr. Miller asked whether any of these laboratories have assayed for helper virus. Dr. Bank said that approximately 20 of the 300 laboratories had tested for helper virus. Dr. Miller said that experiments must be performed with the proposed packaging cell line and vector in order to demonstrate that helper virus has not been produced. With

regard to the vector titer, Dr. Bank stated that the titer is approximately 5×10^4 as assayed by colchicine resistance; however, this number may be an underestimate.

Dr. Parkman noted that the eligibility criteria for patients who would receive Taxol treatment is not clearly stated in the informed consent document. Dr. Bank responded that he would clarify this section of the informed consent document.

Dr. Leventhal noted that patients will have 3×10^8 ABM cells harvested; 1×10^8 of these cells will be transduced. What procedures will be followed if the proposed number of cells are not obtained in the original harvest? Dr. Hesdorffer answered that if 3×10^8 ABM cells are not obtained then either a lower number of cells will be transduced or no cells at all.

Dr. Bank presented additional data demonstrating an increase in gene expression following Taxol administration. PCR amplification showed that the signal changed from negative to positive after Taxol and a 10-fold increase in MDR expression was observed by FACS analysis 8 months post-transduction in the murine model. FACS analysis of human marrow demonstrated that MDR expression in untransduced early cells is negligible. However, 15-20% of the cells demonstrated a log increase in MDR expression following transduction and 5 days in culture.

Dr. Bank introduced his co-investigator, Dr. Steve Goff, to respond to the committee's questions regarding the vector sequences. Dr. Goff explained that the additional sequences at the end of the vector are derived from the Harvey sarcoma virus genome which has part of VL30 and *env* before the LTR. None of this portion of the vector contains open reading frames. Dr. Post noted that during the review of Dr. O'Shaughnessy's protocol, there was some confusion about the frame shift at the end of the MDR cDNA. Can you assure the RAC that your vector does not contain the same frame shift? Dr. Bank stated that his vector does not contain a frame shift at the end of the MDR cDNA.

Dr. Parkman stated that approval of this protocol should be deferred because it lacks necessary information. There are basic requirements that a protocol should meet prior to submission: (1) investigators should demonstrate the lack of replication competent virus with the proposed vector. The minimum standard for RCR testing is the $S^+ L^-$ assay. (2) transduction and expression should be demonstrated using the proposed vector and target cell. Transduction does not always have to be performed in an animal model; *in vitro* long-term cultures may also be acceptable. (3) the clinical protocol must include extensive detail regarding all proposed procedures and assays. This protocol does not meet any of the aforementioned criteria.

Committee Motion

A motion was made by Dr. Parkman and seconded by Ms. Grossman to defer approval of the protocol. There was a brief discussion between RAC members in which they agreed that Dr. Bank had not demonstrated transduction in the reconstituting cell population that would ultimately be administered to the patient. Although transduction data was submitted using the murine stem cells, the same transduction protocol was not used. Dr. Bank noted that he has successfully transduced CFU-GEMM cells. Dr. Leventhal said that the CFU-GEMM data was not submitted to the RAC. Dr. Parkman stated that additional data should have been submitted demonstrating the effect of the proposed dose of Taxol following ABM transplantation. Safety must be demonstrated with the proposed vector. Dr. Leventhal stated that data should be obtained demonstrating transduction of CD34(+) cells in a "macro" system.

Dr. Michael Gottesman, a co-investigator, commented that there is an implicit assumption that the MDR gene has to be inserted into stem cells in order to provide protection. Short-term protection by active cells might be equally as effective in providing protection against the chemotherapeutic agents as stem cells; particularly since stem cells already exhibit a slight degree of MDR protection. One of the goals of this protocol is to determine whether the MDR gene will be inserted into stem cells. Therefore, failure to demonstrate transduction of stem cells is not a valid reason for disapproval of this protocol. Dr. Parkman stated that the objectives of the protocol had not been presented in this manner. If the protocol was designed as a trafficking study to determine which cell populations are transduced by this procedure, the RAC may have approved the study as a legitimate protocol. The protocol has not been written with this intent. Dr. Leventhal agreed with Dr. Parkman and noted that endpoints are the real issue. If stem cell transduction is demonstrated, the patients could probably receive several courses of Taxol. If committed progenitor cells are transduced, patients may require ABM transplantation after each course of Taxol.

Approval of the protocol is deferred until the investigators provide the following additional information for full RAC review: (1) safety data, e.g., S⁺ L⁻ assays demonstrating the absence of RCR using the proposed vector, PHaMDR1/A and the amphotropic retrovirus packaging line, GP+envAM-12; (2) toxicity data demonstrating the effect of Taxol, at doses comparable to those proposed in the protocol, in patients undergoing ABM transplantation; and (3) transduction of CD34(+) bone marrow cells in a large animal model or long-term bone marrow culture.

The motion to defer approval of the protocol was approved by a vote of 20 in favor, 0 opposed, and no abstentions.

V. **ADDITION TO APPENDIX D OF THE NIH GUIDELINES REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: ADMINISTRATION OF NEOMYCIN RESISTANCE GENE MARKED EBV SPECIFIC CYTOTOXIC T LYMPHOCYTES TO**

**RECIPIENTS OF MISMATCHED-RELATED OR PHENOTYPICALLY SIMILAR
UNRELATED DONOR MARROW GRAFTS/DRS. HESLOP, BRENNER, ROONEY**

Review--Dr. Straus

Dr. Walters called on Dr. Straus to present his primary review of the protocol submitted by Drs. Helen E. Heslop, Malcolm K. Brenner, and Cliona Rooney of St. Jude Children's Research Hospital, Memphis, Tennessee.

Dr. Straus stated that the objective of the proposed study is to mark Epstein Barr Virus (EBV)-specific cytotoxic T lymphocytes (CTL) with the gene encoding neomycin resistance (neo^R). The rationale for this protocol is based on the fact that recipients of mismatched related or unrelated bone marrow cells are at a substantial risk for developing EBV-associated lymphoproliferative disorders. The investigators propose to obtain EBV-specific CTL from the donor, transduce these cells with a retrovirus vector encoding neo^R, and adoptively transfer the transduced cells to the recipient. The recipient will be monitored for reconstitution of the EBV-specific CTL response, which would prevent the outgrowth of EBV-transformed T cells and the persistence of marked donor cells. The investigators have demonstrated their ability to generate EBV-specific CTL with a 5-25% transduction efficiency.

Intravenous administration of transduced CTL will be initiated day 45 post-bone marrow transplantation and continued weekly for a total of 4 weeks. Three groups of patients representing 3 to 6 patients per group will receive one of the following dose-escalations: (1) 1×10^7 to 5×10^7 cells, (2) 5×10^7 to 1×10^8 cells, or (3) 1×10^8 to 5×10^8 cells.

Survival of the neo^R CTL and the level of EBV-specific DNA from peripheral blood and saliva samples will be monitored by PCR amplification. Immunologic responses will be monitored *in vitro* by CTL assays and lymphocyte phenotyping. Dr. Straus stated that the investigators have extensive experience with all aspects of the proposed study, and that they have addressed the reviewers questions and comments adequately.

Review--Dr. DeLeon

Dr. DeLeon noted that the investigators had satisfactorily responded to her comments regarding the informed consent document and recommended that the protocol be approved.

Review--Dr. Carmen

Dr. Carmen stated that this protocol is very well designed. In particular, the investigators have provided a discussion about the inappropriateness of using the *herpes*

simplex-thymidine kinase/ganciclovir system for this study. The comparison of methodologies that was included in the submission was informative.

Other Comments

Ms. Meyers stated that the informed consent document is very well written and that her comments are minor. The parent and patient informed consent documents should include the statement, "The patient will not derive any personal benefit from this protocol; however, knowledge will be gained which will benefit others." The document should include a statement regarding patient follow-up. All gene transfer patients should be followed for life and suggested that the NIH establish a patient registry. A request for autopsy in the event of accidental death should be included in the parent informed consent document. Statements should be included about financing, confidentiality, access to records, and statements about contraception and pregnancy, i.e., for teenagers.

Dr. Zallen responded to Ms. Meyers comment about the establishment of a gene transfer registry. Dr. Zallen inquired about an appropriate course of action that the RAC could take regarding the establishment of gene transfer registry. Dr. Walters reminded the RAC members that Dr. Fred Ledley, Texas Medical Center, Houston, Texas, previously submitted a proposal to the RAC regarding a human gene transfer patient registry for information purposes. Dr. French Anderson, University of Southern California, Los Angeles, California, stated that he has worked with Dr. Ledley for approximately 10 years in an effort to obtain financial support for a patient registry. However, because of the current budget cuts, the registry may not be supported by the NIH. Dr. Leventhal said that it is not appropriate to discuss the future of a patient registry during the discussion of Dr. Heslop's protocol, and the RAC should focus their comments on the EBV-specific CTL study.

Dr. Walters inquired as to whether this protocol is considered a therapeutic or a trafficking study. Dr. Heslop responded that this protocol is a Phase I study; therefore, efficacy will not be determined. Dr. Heslop stated that although it would be encouraging if an effect was observed from the adoptive transfer of these EBV-specific CTL, this protocol is solely a marking study.

Dr. Leventhal explained that human gene transfer and therapy protocols should be categorized by the function of the gene that is inserted. Since the gene transferred for this study encodes for neo^R, which has no therapeutic potential, this protocol should be considered gene transfer.

Committee Motion

A motion was made by Dr. DeLeon and seconded by Dr. Leventhal to approve the

protocol. The protocol was approved by a vote of 19 in favor, 0 opposed, and no abstentions.

VI. ADDITION TO APPENDIX D OF THE NIH GUIDELINES REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: *ASSESSMENT OF THE EFFICACY OF PURGING BY USING GENE-MARKED AUTOLOGOUS MARROW TRANSPLANTATION FOR CHILDREN WITH ACUTE MYELOGENOUS LEUKEMIA IN FIRST COMPLETE REMISSION*/DRS. BRENNER, KRANCE, HESLOP, SANTANA, AND IHLE

Review--Dr. Chase

Dr. Walters called on Dr. Chase to present his primary review of the protocol submitted by Drs. Malcolm K. Brenner, Robert Krance, Helen E. Heslop, Victor Santana, and James Ihle of St. Jude Children's Research Hospital, Memphis, Tennessee.

Dr. Chase explained this is a marking study in which two different retrovirus vectors, LNL6 and G1Na, will be used to compare the efficacy of two bone marrow purging techniques, 4-hydroxyperoxycyclophosphamide (4-HC) and interleukin-2 (IL-2) activation of cytotoxic effector cells. Data from previous studies indicates that gene-marked malignant cells have been identified in the marrow of remission patients and can contribute to recurrent disease. Therefore, the current purging methods probably do not eliminate all of the malignant cells in the reinfused marrow.

Review--Dr. Doi (presented by Dr. Chase)

In Dr. Doi's absence, Dr. Walters asked Dr. Chase to summarize Dr. Doi's written comments regarding this protocol.

Dr. Chase noted that the investigators had responded to all of Dr. Doi's written comments and questions. Dr. Doi had inquired whether it was possible to determine the percentage of relapsed cells that contained marker genes in the 2 marked relapse patients from the previous study. The investigators' response to this question was that 2% of the relapse cells were marked; however, since only one-third of the reinfused marrow was transduced, the total number of marked cells could be as much as 6%.

Dr. Chase commended the investigators for their responses regarding the patient sample size. A detailed discussion and published article about the sample size and power computations has been included. Dr. Chase suggested that all investigators should submit an analysis section according to these standards. He complemented Dr. Brenner on his clear explanation of the issues and recommended that the protocol be approved.

Review--Ms. Meyers

Ms. Meyers stated that this protocol is the logical next step in a series of protocols that have been initiated by the investigators. The initial concerns about the informed consent document have been adequately responded to, except for the addition of a request for autopsy in the parent consent form. Dr. Brenner had stated that it is the policy of St. Jude Children's Research Hospital to verbally notify all patients and their parents that an autopsy will be requested in the event of death; however, this statement is not included in the informed consent document. Ms. Meyers stated that it is important that this statement be reflected in the parent consent form.

Other Comments

Dr. Parkman inquired whether the IL-2 purging procedure will be performed in Vancouver; and if so, will the bone marrow cells be shipped? If the cells are shipped, what are the risks involved? In addition, there will be a 6 hour delay between cell isolation and 4-HC purging due to the transduction procedure. Is the sensitivity of the leukemic or normal cells to 4-HC affected by the long incubation period?

Dr. Post asked the investigators to provide information about the patients who have received transduced cells and not relapsed on their previously approved protocols. Do they have marked cells? Do you have data regarding the efficacy of transduction, etc.?

Dr. Haselkorn noted that other investigators have utilized other purging methods such as magnetic bead/antibody selection procedures. Why has 4-HC and IL-2 activation been chosen over other selection procedures?

Presentation--Dr. Brenner

Dr. Brenner stated that 12 patients will be entered onto this protocol per year. At the end of each year the results of the protocol will be assessed. The ongoing study was initiated in September 1991, and 12 patients have been entered onto the protocol. Two of these patients have relapsed; both of these have been marked. In addition, a third patient has relapsed as demonstrated by cytology; however, this patient has not been clinically determined to be in relapse. A maximum number of 35 patients will be enrolled only if no marked relapses have been observed. A significant number of marked relapses will indicate reconsideration of the protocol.

In response to Ms. Meyers' concerns about the request for autopsy in the informed consent document, Dr. Brenner said that he did not believe that the St. Jude's IRB will ever approve such a statement. Dr. Brenner noted that there have been 4 patient deaths to date that have not been associated with the gene marking procedure, and that all 4

patients underwent autopsies at the time of death. This standard request is entered into the patient's chart at the time the family is contacted.

Ms. Meyers asked if all of the deaths occurred at St. Jude. Dr. Brenner responded that 3 of the 4 patients died while at St. Jude, and that all of the autopsies were performed at St. Jude.

Dr. Brenner explained that the IL-2 purging procedures will be performed in Vancouver. Four bone marrow preparations have been shipped back and forth from St. Jude to Vancouver, and the cells have remained viable. After the cells are purged in Vancouver, they will be sent back to St. Jude to be grown in culture for 7 days and then cryopreserved. The purged cells have retained their viability upon thawing.

Dr. Parkman inquired if any patients have received ABM transplantation with IL-2 purged cells that have not been gene-marked. Dr. Brenner answered that no patients have received IL-2 purged ABM cells at St. Jude; however, 14 patients have undergone this procedure at another institution. Dr. Brenner stated that there is no evidence that the sensitivity of these cells to 4-HC is altered following the 6 hour incubation period.

Dr. Brenner stated that all of the patients who have not relapsed are evaluable for engraftment. Of these 18 patients, 16 demonstrated marked cells which are hematopoietic progenitor cells, lymphoid cells, or a combination of both. The level of marking ranges between 1 and 12%, which is much greater than was originally anticipated.

Dr. Brenner explained that the reason that the magnetic bead separation procedures have not been used for these studies is that the technique requires an antibody that specifically recognizes the leukemic cells. These cells do not usually possess a unique phenotype. The 4-HC and IL-2 purging methods were chosen because effective purging will probably prove to be a combination of these two techniques. To date, the most successful treatment of leukemia has proven to be a combination of therapies which do not cause excessive toxicity.

Dr. Parkman asked Dr. Brenner whether the St. Jude IRB was concerned that the shipment of cells to Vancouver imposed any increased risk to the patient. Dr. Brenner said that two-thirds of the marrow remains cryopreserved at St. Jude; therefore, any unforeseen loss of the shipped cells should not affect engraftment of the untransduced cells. Dr. Brenner agreed; however, that the shipment issue should be included in the informed consent document.

Dr. Post noted that all patients will receive both purging procedures. He inquired if there would be any additional benefit if the patients were divided into groups that

received only a single treatment of either 4-HC or Il-2. Dr. Brenner responded that although the patients may benefit from a single treatment, it is unknown which is the most efficacious method. There is no data demonstrating that either of these treatments alone or in combination is effective. Dr. Leventhal said that parallel studies with single vectors and purging methods would require a larger number of patients than this simultaneous labelling experiment.

Dr. Leventhal asked how the investigators are interpreting the data which demonstrates 80% gene marking in patients who are still in remission. Dr. Brenner responded that they are transducing a long-lived progenitor cell, if not a pluripotent stem cell. This assumption is based on GEMM colonies growing out at 1 year as well as marking of T and B cells. Four of these patients have an A21 translocation. Other patients have demonstrated CD34 and CD 56 double positive cells. In the neuroblastoma study, 2 patients have relapsed, 1 of which was marked. Dr. Parkman asked if the neuroblastoma relapse was at the original site of disease. Dr. Brenner said that biopsies are currently being analyzed; therefore, the only known site of relapse at this time is the marrow.

Committee Motion

A motion was made by Dr. Chase and seconded by Dr. Haselkorn to approve the protocol. The protocol was approved by a vote of 17 in favor, 0 opposed, and no abstentions.

II. ADDITION TO APPENDIX D OF THE NIH GUIDELINES REGARDING A HUMAN GENE THERAPY PROTOCOL ENTITLED: *A PHASE I TRIAL OF HUMAN GAMMA INTERFERON-TRANSDUCED AUTOLOGOUS TUMOR CELLS IN PATIENTS WITH DISSEMINATED MALIGNANT MELANOMA/DR. SEIGLER*

Review--Dr. Leventhal

Dr. Walters called on Dr. Leventhal to present her primary review of the protocol submitted by Dr. Hilliard F. Seigler of Duke University Medical Center, Durham, North Carolina.

Dr. Leventhal provided a brief overview of the proposed study. This protocol is a Phase I trial of human gamma interferon (γ -IFN)-transduced autologous tumor cells in patients with disseminated malignant melanoma. Autologous tumor cells will be grown in short-term culture and transduced with the gene encoding for γ -IFN. Patients will receive subcutaneous injections of 3×10^7 transduced irradiated tumor cells every 2 weeks for 3 months and 1 injection per month for a total of 24 injections.

Animal data has been submitted demonstrating that transduced cells grow at a slower

rate than untransduced cells, and that a CTL response is generated. The investigators propose that the delayed growth rate of the tumor cells is a secondary effect of the CTL. Dr. Leventhal noted that her written comments to the investigators referred to the fact that the data did not demonstrate a diminution or maintenance of tumor growth in an immune-competent animal. The data submitted by Dr. Seigler showed that γ -IFN transduced B16 melanoma cells grow more slowly than untransduced tumor cells in nude mice. Dr. Leventhal stated that the murine *in vivo* data suggests that the slowed growth rate of the tumor cells may not be due to the generation of CTL, but to natural killer cells or some other mechanism.

Based on this *in vivo* data, there is concern about the specific parameters that will be monitored in terms of patient response, i.e., the generation of CTL. The investigators have not provided data demonstrating that the injection of transduced tumor cells at 1 site affects the growth of untransduced tumor cells at other sites. Although the murine data suggests that transduced cells prevent the occurrence of tumor at other sites when injected intravenously, they have not demonstrated any effect on established tumor nodules.

The investigators have agreed that patients must have measurable lesions following the removal of the tumor cells for transduction. Dr. Leventhal said that she had requested that a statement be included in the informed consent document which explains to the patient that tumor cells will be removed in an attempt to perform this experiment; however, there is a possibility that they may not receive any injections.

The endpoints of this study have not been well-defined. The investigators have stated that toxicity will be the endpoint; it is unclear how toxicity is defined. They state that the development of a granuloma or other vigorous immune response at the site of the injection is the desired endpoint. If the generation of CTL will be used as the endpoint, the animal data is insufficient to justify this evaluation criterion.

Review--Dr. Parkman

Dr. Parkman said that he was concerned about the lack of data demonstrating an anti-tumor effect on established tumor. The investigators have indicated that tumor-specific CTL can be induced by *in vitro* stimulation of peripheral blood lymphocytes (PBL) using autologous or human leukocyte antigen (HLA)-matched tumors. However, the data which was submitted was generated using a cell line. In addition, there is no independent control included to demonstrate the nonspecific effect of γ -IFN.

Dr. Parkman stated that his written review asked if there is a minimum amount of γ -IFN that is necessary to achieve the desired effect. The investigators answered that a minimum of 2 units of γ -IFN/ 1×10^6 cells/day would be required. Dr. Parkman noted

that the investigators did not provide a dose-response curve comparing the amount of γ -IFN to the induction of Class II expression, which is one of the proposed mechanisms of the anti-tumor response.

Review--Ms. Meyers

Ms. Meyers stated that the revised version of the informed consent document submitted by the investigators is much improved over the original; however, it still does not include a request for autopsy. The investigators should describe the financial obligation of the patient for the treatment of any adverse effects which might possibly result from the gene transfer procedure.

Other Comments

Dr. Zallen said that the informed consent document is poorly written. When the tumor cell transduction process is described, the investigators should explain that this procedure will be conducted *in vitro*, not *in vivo*. This section should also include a statement explaining that if the *in vitro* procedure is unsuccessful, then the patient will not receive any cell injections. The *Points to Consider in the Design and Submission of Protocols for the Transfer of Recombinant DNA into the Genome of Human Subjects (Points to Consider)* should be clarified with regard to potential benefits and possible adverse reactions. The investigators should explain the entire informed consent process at Duke University Medical Center.

Dr. Post asked the investigators to provide further information about the proposed vector and packaging cell line. Is there homology and overlap between them? What is the investigators' experience with this vector and this packaging cell line?

Dr. Miller presented a brief description of the proposed vector. This vector has a γ -IFN coding sequence and a mutated *gag* start codon, ATT, which prevents *gag* translation. There is an LTR, a packaging signal that contains portions of *gag*, and the γ -IFN gene. Then there is an SV40 neo^R sequence at the 3' end that will be used for selection purposes.

Dr. Miller said that one possible concern is that this vector produces a protein that is made in the *gag* region upstream from the CTG start codon, or glycosylated *gag* protein, which has been altered in other vectors previously been approved by the RAC. It is unknown what effect this protein would have on the protocol. The investigators have provided safety studies using this vector which indicate that this extra open reading frame should not be a serious concern.

Dr. Miller said that one of the packaging cell lines that is mentioned in the protocol has

been demonstrated to generate helper virus in the past; however, the investigators will probably propose to use another version of that packaging cell line which is more contained. The RAC must keep in mind that any packaging cell line will generate helper virus at some rate, and that the breakout that occurred was a nonhomologous recombination. There was no overlap in the *env* region between the vector and the packaging cell line.

Dr. Anderson stated that the precise mechanism of the breakout is unclear; the issue of homologous versus nonhomologous recombination has not been absolutely determined. Dr. McGarrity stated that if homologous recombination occurs, it will probably occur within the vector and packaging system. There is an overlap in the *gag* region of approximately 50 base pairs; however, there is no overlap in the *env* region. Dr. Geiduschek stated that although the recombination event should not be categorized prematurely, it is extremely important to understand the mechanisms of these rare events in order to gain an understanding of the risks. When the mechanism of recombination is determined, the RAC should be notified of this information immediately.

Presentation--Dr. Seigler

Dr. Seigler responded to the RAC members questions regarding the eradication of established tumor in the murine model. The murine experiments have not been performed because they do not accurately represent the human situation. Murine and human γ -IFNs are species-specific; therefore, the research has focused on the human situation.

Data was presented demonstrating decreased tumor growth in response to transduced B16 melanoma cells in immune-competent mice. Additional data indicated that a decrease in the growth rate of L33 tumor cells was due in part to CTL based on the fact that tumors do not develop in normal mice but do occur in nude mice.

Dr. Seigler stated that endpoints will be determined by two measurements: (1) tumor status, and (2) the generation of CTL. Patients with stable disease will remain on the study, and these who demonstrate progressive disease will not continue in the protocol. If 10 patients demonstrate no CTL response, the study will be terminated unless there is clinical evidence of tumor response, i.e., stable or regressing tumor.

Dr. Parkman asked if there are data demonstrating CTL responses from autologous PBL to tumor cells. Dr. Seigler said that CTL responses were generated *in vitro* to tumor cell lines. Dr. Parkman stated that established cell lines are very different from autologous fresh tumor cells. Dr. Darrow of Duke University Medical Center, Durham, North Carolina, stated that the induction of melanoma-specific CTL has been demonstrated with both transduced and untransduced cells against tumor cell lines and fresh

autologous tumor cells. Transduced tumor cells demonstrate a significant increase in the level of cytolytic activity. Dr. Parkman said that this data should have been provided to the RAC. Dr. Darrow said that these CTL responses have been melanoma antigen restrictive. Dr. Darrow said that he regrets that this data had not been included in their submission. As a point of clarification, Dr. Leventhal asked whether PBL from tumor-bearing patients demonstrate greater cytolytic activity against transduced autologous tumor cells than untransduced tumor cells. Dr. Darrow said that data indicates these transduced cells do generate a greater level of CTL activity than untransduced cells.

With regard to Ms. Meyers's concern about the informed consent document, Dr. Seigler said that the document now states, "However, if incorporation of the virus into the tumor does not occur you will not be able to continue with the study." This statement along with the inclusion of *in vitro* transduction should provide clarification. Ms. Meyers suggested that the term "laboratory setting" be used in place of *in vitro*. Dr. Seigler agreed to the suggested change.

Dr. Seigler stated that the patient will not be responsible for any of the costs associated with the experimental procedures. All other costs, including costs associated with treatment, will be the responsibility of the patient and/or the third-party insurance carrier. Immediate medical care will be provided to the patients in the event that injury occurs as a result of participation in the protocol. However, there is no provision for free medical care or monetary compensation for such injury.

The informed consent process involves the PI, the patient, a family member, and one other neutral party, i.e., a nurse clinical specialist. Dr. Zallen inquired about the time frame that is involved in the informed consent process. Dr. Seigler stated that the patient has a minimum of 1 full day to consider the informed consent document and usually has several weeks to reconsider their decision before the procedure is initiated.

Discussion

Dr. Parkman asked the investigators if there are data demonstrating that 2 units of γ -IFN is adequate to induce Class II expression. Dr. Seigler stated that 2 units is a minimum level of production. Of 5 tumor cell lines that have been transduced, there is an average of 45 units of γ -IFN produced per 1×10^6 cell per day. Dr. Parkman asked Dr. Seigler what he would do if a patient's transduced cells produced only 2 units of γ -IFN/ 1×10^6 cells/day. Dr. Seigler said he would not inject cells that demonstrated this minimum level of γ -IFN expression. Dr. Parkman stated that the investigators should provide a dose-response curve demonstrating γ -IFN production verses Class II expression.

Dr. Parkman inquired if $S^+ L^-$ assays have been performed. Dr. Seigler said that these

assays have been performed, and that Dr. Jack Barber of Viagene, Inc., would provide a summary of these data. Dr. Leventhal asked what would be considered as dose-limiting toxicity in these patients. Dr. Seigler responded that toxicity is no longer being considered as an endpoint. The endpoints will be tumor and CTL responses. In addition, if no CTL activity is demonstrated after 10 patients, the study will be terminated unless tumor regression is observed.

Dr. Leventhal asked how toxicity will be monitored and evaluated as a stop criterion. If a patient demonstrates fever, chills, and shock, there must be a point at which the protocol will be terminated. Dr. Seigler agreed to include the suggested stop criteria in the protocol.

Ms. Meyers asked if the patient would be liable for any costs associated with adverse effects associated with an overdose of γ -IFN. Dr. Seigler stated that the patient would not be financially responsible for the cost of treatments that resulted directly from the research.

Dr. Haselkorn inquired about the responsibilities of the co-investigators and if Dr. Seigler is the only investigator who would have contact with the patient. Dr. Jolly of Viagene, Inc., responded that he and Dr. Barber will produce the vector at Viagene; Drs. Seigler and Darrow will perform the laboratory experiments at Duke University. Dr. Seigler will have direct contact with the patients.

Dr. Barber of Viagene, Inc., addressed the RAC members' questions regarding the vector and packaging cell line. The proposed vector and packaging cell have not produced helper virus breakout; the breakout that the committee members referred to was with another packaging cell line. The proposed system has been extensively characterized. All of the RCR testing will be repeated after the master cell bank and working cell banks have been established. The testing of the cell banks is currently in progress.

Dr. Post asked further questions regarding the helper virus breakout. If the breakout did not occur with the proposed packaging cell line and virus, did it occur with the same packaging cell line and another virus? Dr. Barber stated that the breakout occurred with a different virus and a different packaging cell line. Dr. Post asked about the difference between the two systems. Dr. Barber explained that the gene expression vectors are the same; however, everything else is different. The cell line that had the breakout of helper virus was the canine fibroblast cell line, CF2. The current packaging cell line is based on the canine cell line, D17. The only similarity between the 2 systems is the expression of the Moloney structural gene products. Dr. Barber noted that the reason that the RCR safety data had not been included in the original submission was that the master and working cell banks had not yet been established.

Dr. Leventhal said that approval of this protocol should be deferred until the investigators submit data demonstrating the following: (1) regression of established tumor, (2) generation of CTL from human PBL from tumor-bearing patients in response to autologous tumor, (3) a quantitative correlation between Class II antigen expression and the amount of γ -IFN, and (4) a quantitative correlation between the number of CTL generated per unit of γ -IFN.

Dr. Barber stated that it would not be appropriate to use the human vector in the animal model system because γ -IFN is species-specific. Dr. Leventhal suggested that quantitative data should be submitted demonstrating the correlation between the generation of CTL from human PBL and the amount of human γ -IFN produced. In addition, the investigators should identify a safe threshold of minimal expression. Dr. Leventhal said that this *in vitro* data would be acceptable in place of the *in vivo* data.

Dr. Leventhal stated that she is not convinced that there is sufficient justification to conduct this protocol based on the data that was submitted. Dr. Parkman said that the important issue is whether the RAC should approve every protocol in which a cytokine gene is inserted into melanoma cells. There has to be evidence that the cytokine gene augments the immune response.

Presentation--Dr. Darrow

Dr. Darrow presented additional data to the RAC which was not included in the meeting materials. Blastogenesis data indicated a substantial increase in thymidine incorporation in response to γ -IFN transduced tumor as compared to untransduced tumor. Dr. Parkman noted that the data was derived using established tumor cell lines. Dr. Darrow said that the cell line was derived from a melanoma patient, and that it had been cultured for a maximum of 5 weeks. Dr. Darrow presented a representative experiment with several different cell lines which demonstrated increased efficacy of CTL generation with γ -IFN transduction. Dr. Darrow stated that these assays are performed after 4-5 weeks in culture, because CTL activity peaks at this time and nonspecific killing is absent. This *in vitro* data substantiates the hypothesis that transduced cells induce a significant increase in the level of CTL activity; therefore, a similar response should be achieved *in vivo*.

Discussion

Dr. Leventhal said that the data is insufficient to satisfy her concerns. Data must be derived from multiple experiments and statistically analyzed. Dr. Miller said that he would recommend approval of the protocol contingent on the submission of additional data. Dr. Leventhal stated that contingent approval of this protocol would not be consistent with the standards that have been used for other investigators.

Dr. Miller said that most protocols have been deferred based on the lack of safety data about the proposed vector and packaging cell line. Dr. Seigler has demonstrated safety; however, he is lacking data regarding the immune response. Dr. Miller said that if the RAC members insist that the investigators submit the required data, they should be allowed the opportunity to submit the data in response to a contingent approval. Dr. Motulsky agreed with Dr. Miller stating that the RAC should not stand on ceremony and precedence.

Dr. Haselkorn asked the RAC members to specify the exact information that should be submitted. Dr. Miller said that the CTL response data should be determined to be statistically significant. Dr. Geiduschek said that Dr. Motulsky is making the assumption that the RAC would be completely satisfied with the submission of additional data. Dr. Leventhal said that it is in the investigators' best interest to resubmit their data for full RAC review.

Committee Motion

A motion was made by Dr. Leventhal and seconded by Ms. Meyers to defer approval of the protocol. Dr. Miller asked the investigators if they had an anticipated time frame in which the vector would be available for transduction. Since the master and working banks have not been established and tested, additional review at the June RAC meeting should not significantly delay the process. Dr. Jolly responded that an additional 3 months could introduce a slight delay. Dr. Leventhal reminded the investigators that the Food and Drug Administration (FDA) and RAC approvals are parallel processes; therefore, a delay in RAC approval will not affect their ability to obtain FDA approval. Dr. Miller asked the investigators if they have submitted their Investigational New Drug (IND) application to the FDA yet. Dr. Seigler responded that they had not yet submitted their application to the FDA.

The motion to defer approval of the protocol was approved by a vote of 16 in favor, 1 opposed, and 1 abstention. Approval of the protocol was deferred until data is submitted for full RAC review of the following: (1) generation of CTL from human PBL from tumor-bearing patients in response to transduced autologous tumor, (2) quantitative correlation between Class II antigen expression and the amount of γ -IFN, and (3) quantitative correlation between the number of CTLs generated per unit of γ -IFN.

VIII. ADDITION TO APPENDIX D OF THE NIH GUIDELINES REGARDING A HUMAN GENE THERAPY PROTOCOL ENTITLED: *PHASE I STUDY OF NON-REPLICATING AUTOLOGOUS TUMOR CELL INJECTIONS USING CELLS PREPARED WITH OR WITHOUT GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTOR GENE TRANSDUCTION IN PATIENTS WITH*

METASTATIC RENAL CELL CARCINOMA/DR. SIMONS

Review--Dr. Smith

Dr. Walters called on Dr. Smith to present his primary review of the protocol submitted by Dr. Jonathan Simons of Johns Hopkins Oncology Center, Baltimore, Maryland.

Dr. Smith provided a brief overview of the proposed study. This protocol is a Phase I vaccination study for the treatment of metastatic renal cell carcinoma for patients who have failed conventional therapy. Patients will receive transduced or untransduced cells in a randomized dose-escalation fashion.

The investigators have submitted extensive data from an *in vivo* murine model. The rationale for this protocol is based on published scientific data demonstrating that granulocyte-macrophage colony stimulating factor (GM-CSF) significantly enhances tumor regression. Safety and tolerance of the vaccination procedure, as well as any adverse effects associated with gene transfer, will be critically evaluated. Long-term potential toxicity will also be monitored, i.e., autoimmune disease. In addition, the investigators will monitor immune response to the transduced and untransduced cells.

The proposed retrovirus vector has a high efficiency of transduction and does not require selection. The patient's cells will be irradiated with a maximum dose of 15,000 rads. This dose of radiation does not adversely affect GM-CSF production, but inhibits tumor cell growth. Primary cell cultures will be used instead of long-term cell culture lines.

Dr. Smith stated that his written comments to the investigators addressed their ability to evaluate immune responsiveness in these patients in the absence of clinical endpoints. The investigators provided adequate responses and explained that additional biopsies of the draining lymph nodes potentially will be taken and assayed for immune responsiveness. The investigators should describe the types of assays that will be performed on these biopsy specimens. Also, the investigators should provide additional data regarding the safety and design of the proposed vector.

Review--Dr. Brinckerhoff

Dr. Brinckerhoff stated that this protocol is very well designed and documented. The major concern is the investigators' ability to cultivate and grow the primary tumor cell cultures obtained from the patients' kidneys. Primary cell cultures are difficult to initiate and expand. The investigators responded that they have been successful in all except one attempt to develop these primary cell cultures. The investigators should comment on the ease with which they have been able to establish these cultures.

Review--Dr. Carmen

Dr. Carmen stated that this protocol is innovative and fascinating. The investigators should describe the differences between transduced and untransduced irradiated cells, and their ability to elicit an immune response. The investigators need to explain the differences between the MFG and MFGS vectors. Dr. Carmen said that this protocol is very interesting and recommends that it be approved.

Other Comments

Ms. Meyers provided comments about the informed consent document. The document should contain an explanation of gene therapy, including a lay description of vectors and the gene transfer process. A statement should be included about patient confidentiality, and that the NIH and the FDA will have access to the patient's records. A section should be included about the time frame for patient follow-up and the procedures involved. The informed consent document provides a recommendation to women regarding the use of contraception; however, this statement should include men. A statement should be added about a request for autopsy.

Dr. Miller provided a description of the proposed vector. This vector is based on the N2 vector which has been previously reviewed by the RAC. The vector contains the *gag* region of the Moloney murine leukemia virus (M-MuLV) at the 5' end and the *env* sequences of the parental virus at the 3' end. When used in combination with the packaging cell line, there is the potential for homologous recombination and the generation of helper virus. One of the problems associated with an N2 based vector is the large *gag* open reading frame. Since the *gag* protein is antigenic, the effects of this protein must be considered.

Dr. Miller explained that the investigators have proposed a new vector, MFGS, in which deletions and mutations have been incorporated to eliminate *gag* protein synthesis. However, the RAC must consider that the animal data that has been submitted was generated using the MFG vector, not MFGS. The committee should be certain that the new animal data, generated with the new vector, correlates with that of the old vector. MFGS differs from MFG in that a cryptic splice has been included upstream from *gag*; therefore, this vector is not new. MFGS is still an N2 based vector. Past experience has demonstrated that it is very easy to generate helper virus from N2.

Dr. Miller noted that the LN series was developed in response to the helper virus problems associated with N2. In the LN vectors, the *env* region was deleted in order to eliminate the possibility of homologous recombination. The investigators have not detected any helper virus associated with the use of the MFG vector; however, there is the potential for helper virus production. The MFGS vector has a mutated *gag* region;

however, the *env* remains which could allow for homologous recombination.

Dr. Haselkorn asked if the RAC is being asked to consider a vector that has not been thoroughly tested. Dr. Miller responded that the proposed vector has been tested extensively; however, the committee should consider the theoretical probability of such an event. Dr. Haselkorn said that it is imperative that the investigators address the issue of helper virus in their responses to the committee.

Dr. Walters noted that two RAC members, Drs. Chase and Leventhal, have recused themselves from reviewing and voting on this protocol because they are employees of Johns Hopkins University, and do not want to present the appearance of conflict of interest. Dr. Walters called on Dr. Simons to respond the questions and comments of the RAC members.

Presentation--Dr. Simons

Dr. Simons introduced his co-investigators: Drs. Richard Mulligan, Glen Dranoff, Larry Cohen, Fray Marshall, William Nelson, Drew Pardoll, Steven Pianadori, Elizabeth Jaffe, Karen Hauda, Jim Zabora, and Beth Gregory. Dr. Simons stated that Dr. Mulligan would address the RAC members' questions and comments about the MFG and MFGS vectors.

Dr. Mulligan said that, in general, he agreed with Dr. Miller's comment that there is no packaging cell line that is absolutely safe. The vector has an open reading frame that expresses the p15 protein. One of the positive features of this vector is that it possesses extra packaging sequences. When the desired sequences are inserted into the vector, the protein which is expressed is very similar to the normal protein and is very efficiently expressed.

In terms of safety, this vector has been tested extensively in the packaging cell line, Ψ -crip. Dr. Mulligan stated that there have not been any concerns about the existence of open reading frames in this vector. He said that 30 separate constructs have been made and that no helper virus production was detected in any case. The assays for the detection of helper virus have been performed in-house as well as by reliable outside laboratories.

Dr. Mulligan stated that they attempted to introduce simple changes that would increase the safety potential of the MFG vector; this safety modified vector is MFGS. A stop codon has been introduced in MFGS in order to inhibit the open reading frame from initiating p15 protein production. The key factors that were considered in evaluating this modified vector as compared to the parent vector were: (1) the efficiency of gene transfer, (2) level of gene expression, and (3) the immune effects observed in relation to

GM-CSF expression.

The modified vector has been used with approximately 30 different cytokines. In every case, the modified vector performed as well as the original vector. Recently, there has been concern among the scientific community that the introduction of multiple antigens may reduce the specificity of the T cell response. In light of these concerns, the absence of the neo^R coding region may offer an advantage. The modifications that have been introduced are simple and offer a theoretical safety advantage over the original vector. Data was presented demonstrating that there are no significant differences in gene transfer or GM-CSF expression between the 2 vectors.

Dr. Post asked whether animals that receive MFG transduced cells develop an immune response to the GM-CSF. Dr. Mulligan said that the immune response to this protein in animals has not been examined in a careful fashion. Dr. Geiduschek asked Dr. Mulligan to describe the particular advantages of the new vector in relation to geometry, concentration, and the detection of helper virus breakout. Dr. Mulligan said that the new vector allows for very high virus titers using relatively low numbers of producer cells. This system allows for the possibility of preserving all of the producer cells in order to test for helper virus generation. These producer cells can be maintained in culture for 7 days.

Presentation--Dr. Simons

Dr. Simons responded to Dr. Smith's questions about the parameters that will be used to monitor immune response. Dr. Simons explained that immune response will not be used as an endpoint for this study since this protocol is Phase I. The immunologic assays outlined in the protocol are merely for information purposes. The assays that will be used to analyze immune function in future studies are still in the developmental stages. The immune parameters have not yet been defined.

In regard to the ability to establish primary cultures from these patients, Dr. Simons explained that Drs. Jaffe and Marshall have developed a special tissue culture medium which allows for the specific expansion of these primary cells. These studies have been performed in collaboration with Somatix Therapy, Inc. Somatix has been responsible for the early feasibility studies involving these primary cultures. Patients' cells have been routinely shipped from Johns Hopkins to Somatix, cultured, and transduced. Dr. Simons said that they were able to culture 11 out of 12 renal cell carcinomas and grow these cultures to sufficient numbers that would make the patients eligible for this study.

Dr. Simons responded to Dr. Carmen's comments about the effect of radiation on both transduced and untransduced cells. When non-irradiated tumor cells are injected, these cells outgrow the inoculation site. The rationale for irradiation is patient safety. Data

demonstrates that irradiated tumor cells are equally effective as non-irradiated cells at evoking an anti-tumor response.

In response to Ms. Meyers' concerns about the informed consent document, Dr. Simons explained that the Johns Hopkins IRB will not approve an informed consent document similar to those approved by the RAC, because the reading level is too high. Johns Hopkins requires the document be worded at a sixth grade reading level. An attempt will be made to incorporate statements about confidentiality, contraception, and request for autopsy at the sixth grade reading level; however, there is no guarantee that the IRB will approve these additions.

Ms. Meyers said the informed consent document that was approved by the Johns Hopkins IRB is outrageous. The language is not written at the sixth grade level. The sentence that explains that the patient will receive an "injection of cancer" is not an accurate description of the procedure that will be performed. Dr. Simons explained that the IRB has very strict standards as to length of the document and the reading level. It is unlikely that the IRB will accept any major revisions or additions to the document.

Dr. Simons explained that the informed consent document is the product of "field testing." The Johns Hopkins IRB asked that the document be distributed to outpatients for comments and suggestions regarding the clarity of the document. The current document has been improved over the original version. Ms. Meyers suggested that the investigators ask the IRB if the document could be expanded to provide a more detailed description of the gene therapy procedures. This protocol is not a drug study but a novel approach which requires further explanation.

Dr. Wivel reminded the members of the RAC that recommendations about IRB informed consent documents are not binding. The IRB has the authority to accept or deny any changes to the document; therefore, the RAC should not include any such contingencies in a motion for approval. Ms. Meyers stated that if a contingency is not included as part of the formal motion for approval of this protocol, she will not vote for approval based on the opinion that patients will be misinformed. Dr. Parkman suggested that changes to the informed consent document should be recommended to the IRB; however, it is inappropriate to disapprove a scientifically sound protocol because the RAC does not approve of the IRB's standards. Dr. Simons stated that he would incorporate the changes suggested by Ms. Meyers, submit these changes to the IRB for approval, and modify the current document to the extent that the Johns Hopkins IRB will allow.

Committee Motion

A motion was made by Dr. Brinckerhoff and seconded by Dr. Carmen to approve the

protocol using the MFGS vector. Approval of this protocol is contingent on the submission of relevant safety studies with the MFGS vector. The motion was approved by a vote of 15 in favor, 0 opposed, and 3 abstentions (Leventhal, Carmen, Meyers).

IX. ADDITION TO THE *POINTS TO CONSIDER* OF THE NIH GUIDELINES REGARDING SEPARATION OF THE GENE MARKING INFORMED CONSENT DOCUMENT FROM THE THERAPEUTIC INFORMED CONSENT DOCUMENTS/DR. POST

Presentation--Dr. Post

Dr. Walters called on Dr. Post to present the proposed amendment to the *Points to Consider*. Dr. Post explained that the proposed amendment is intended to clarify the informed consent process for patients considering participation in a human gene transfer protocol. When one informed consent document is used for two independent procedures the patient may be given the impression that participation in one form of therapy is contingent on participation in the gene transfer protocol. Therefore, a new sentence will be added to Section I-D-Informed Consent of the *Points to Consider*:

"When gene transfer is a procedure separate from the therapeutic protocol, informed consent documents should be submitted for both the gene marking and therapeutic procedures."

Committee Motion

A motion was made by Dr. Post and seconded by Dr. Krogstad to approve the proposed amendment to the *Points to Consider*. Dr. Leventhal objected to the use of the word "marking" and suggested that "transfer" is a more appropriate term because the word "transfer" encompasses marking as well as therapeutic procedures. Also, Dr. Leventhal recommended that the phrase "therapeutic procedures" be replaced with "other clinical protocols." Drs. Post and Krogstad accepted Dr. Leventhal's recommendations as a friendly amendment to the motion.

Dr. Walter stated that the revised language of the proposed amendment will read as follows:

"When gene transfer is a procedure separate from the clinical protocol, informed consent documents should be submitted for both the gene transfer and the clinical protocol."

The motion to approve the revised amendment was approved by a vote of 17 in favor, 0 opposed, and no abstentions.

X. **ADDITION TO THE POINTS TO CONSIDER OF THE NIH GUIDELINES REGARDING THE SAFETY OF DELIVERY/EXPRESSION SYSTEMS AND REPORT ON MURINE RCR ASSAYS/DR. MILLER**

Review--Dr. Miller

Dr. Walters called on Dr. Miller to initiate discussion of the document entitled: *Safety of Delivery/Expression Systems and Report on Murine RCR Assays*, submitted by Dr. W. French Anderson of the Southern California School of Medicine, Los Angeles, and Drs. Gerard J. McGarrity and Robert Moen of GTI, Gaithersburg, Maryland.

Dr. Miller asked if the report was being considered for inclusion in the *Points to Consider* or for endorsement by the RAC as a state-of-the-art guide for investigators about the recommended procedures for RCR testing. Dr. Wivel explained that the report was included as a proposed action to the *Points to Consider* and published in the *Federal Register* to provide the RAC with the option to include specific RCR assay requirements and minimal levels of RCR detection if necessary. Addition of language to the *Points to Consider* by the RAC is an option, not a directive.

Dr. Miller explained that the investigators originally submitted an RCR report to the RAC at the December 1992 meeting. At that meeting, the RAC made several recommendations regarding additions and corrections to the report. This revised report incorporated all of the RAC's suggestions and is a comprehensive summary of murine RCR assays. The report should not be included in the *Points to Consider* due to the rapidly evolving nature of the field. This report should be used as a guide for those investigators who are considering submission of human gene transfer protocols which involve murine retrovirus vector.

Review--Miller (for Dr. Geiduschek)

In Dr. Geiduschek's absence, Dr. Miller summarized Dr. Geiduschek's written critique of the report. Dr. Geiduschek's comments indicated that he is satisfied with the revised version of the report. In particular, he was pleased to see that a section was included that analyzed the dynamics of RCR breakout events. Dr. Geiduschek states that, "The report is to be regarded as a review of status, not as a final resolution of the safety issues." Dr. Geiduschek outlines the following stipulations for endorsement of the report: (1) that the experiment cited on pages 191 and 193 (RAC mailing) are to be repeated sufficiently to establish a time criterion for breakout events, and (2) the fraction of each production lot that will be monitored should be large, i.e., a minimum of 5%. Dr. Geiduschek's written comments indicate that the investigators have agreed to these stipulations, and that these provisions coincide with their own activities and plans.

Dr. Geiduschek recommends that the report be accepted as guidance for investigators, and that it should not be included in the *Points to Consider*.

Presentation-- Dr. Anderson

Dr. Anderson reiterated that fact that the report encompasses ongoing studies, and that these studies are being closely coordinated with the FDA.

Comments--Dr. Gunter

Dr. Kurt Gunter, Acting Deputy Director of the Division of Cell and Gene Therapy, FDA, stated that the FDA agrees with most of the conclusions presented in the report. The one area that the FDA is not entirely in agreement is with the quantitation of risk. The FDA feels that a number of assumptions have been made with regard to this issue and prefers to rely on empirical data rather than assumptions.

During the December 1992 RAC meeting, Dr. Arifa Khan of the FDA presented the FDA's recommendations for a variety of assays for the detection of RCR at various time points in the production process. The reactions of various individuals to the FDA's recommendations illustrates the problems that the FDA has had in dealing with the public. The FDA does not want to outline requirements that are not scientifically founded. However, the FDA is responsible for providing investigators with what is considered the state-of-the-art in terms of RCR testing. As a result, the FDA has devised recommendations that are based on current scientific data and common sense.

The FDA's recommendations are as follows:

- o Master cell bank characterization
 - Supernatant test
 - Co-culture with permissive cell line
- o Manufacturer's working cell bank characterization
 - Repeat test for RCR
 - Test of transduced target cells (high MOI)
- o Release test of production lots
 - Supernatant test
 - Co-culture with permissive cell line

- o Post production run culture and testing
- o Lot release test of transduced cells (*ex vivo*)

Discussion

With regard to Dr. Gunter's presentation, Dr. Post asked if 5% refers to a production lot. Dr. Gunter responded that 5% of the production lot is the recommended testing volume. Dr. Post inquired whether there is any correlation between the size of a production lot and a patient dosage. Dr. Gunter stated that the FDA is not correlating dosage with the size of a production lot at this time. However, as investigators progress to Phase II and Phase III trials and the size of the production lots increases, the FDA will reevaluate these criteria.

Dr. Parkman noted that there are clinical settings in which *ex vivo* testing of transduced cells would not be possible prior to administration, i.e., transduced hepatocytes. There are instances in which either the patient is undergoing a surgical procedure or cell survival is limited *ex vivo*. Dr. Gunter agreed with Dr. Parkman's comments and said that there will be instances where there will be no choice but to proceed with administration of the transduced cells prior to the completion of the proposed assays. Dr. Gunter stated that the FDA will consider any valid, rational, scientific argument against any of the FDA's recommendations.

Dr. Parkman asked about the proposed limit of RCR detection. If one production lot is equal to 5 patient doses, what is the level of detection? Dr. Anderson answered that the limit of detection would be 4 RCR particles. Dr. Miller suggested that the limit of RCR detection should approach 0. Dr. Anderson said that the theoretical limit of RCR detection is 0 particles per production lot, assuming that the lot will be cultured for a sufficient period of time after harvest. However, the practical limits of detection will actually be less than 1 RCR particle per production lot. If a single RCR particle is detected in any assay, the lot will be discarded. Dr. Miller said that the calculations do not accurately reflect the level of detection because the testing volume will be only 5% of an entire production lot. Testing this volume only guarantees that there are less than 20 RCR particles per production lot.

Dr. Chase stated that the calculations should not be interpreted based on sample volume, because this is not a solid object. The probability of contamination is not the same as the fraction of the area that is sampled. Dr. Anderson agreed with the statement made by Dr. Chase and added that carrying a post-production run out for twice the period of time in which an RCR breakout would occur, brings the level of detection to 0 per production lot. Dr. Chase stated that although the desired goal of detection is 0 particles per production lot, you can never be certain that it is absolutely 0.

However, one can approach the 0 particle limit by judicious sampling.

Dr. Gunter stated that the dose of RCR which is disease-causing is still unknown and cited the RCR breakout which resulted in disease in the primate setting, i.e., lymphomas. Until the limits of RCR that cause disease are ascertained, the FDA does not feel that it can define absolute limits for sensitivity of RCR assays. Dr. Gunter said that he is personally uncomfortable with assessing the overall safety of RCR.

Dr. Miller said that Dr. Gunter's statement is not fair to investigators, and that this issue is the basis for which the report was developed. The report provides useful significant detail about the RCR event which occurred in the monkeys and how the monkey situation translates to breakout levels of RCR. Dr. Gunter said that he differs with Dr. Anderson's interpretation because there have been only 5 monkeys that have been tested rigorously for RCR. The monkey database is too small at the current time to draw accurate conclusions.

Dr. McGarrity described a reconstruction experiment designed to reproduce an RCR breakout. After 2 days in culture, the supernatant was removed from 10 RCR producing cells. The cells and the supernatant were then assayed separately for RCR breakout. Breakout was simulated in 2 to 3 days with this method. These studies are currently being refined to achieve a sensitivity level of detecting 1 RCR particle. When assays are perfected at this level, statistical analysis will probably translate into what actually happens when a breakout occurs, i.e., do the number of RCR particles increase tremendously or remain at low levels? These reconstruction experiments are the first attempt to develop a quantitative method for the detection of RCR.

Dr. McGarrity described an RCR breakout that recently occurred in the laboratory setting. The breakout was detected when the RCR titer was approximately 200 particles per ml. This breakout was readily detected by the standard S⁺ L⁻ and NIH3T3 amplification assays.

Committee Motion

A motion was made by Dr. Miller and seconded by Dr. Haselkorn to accept the report as a state-of-the-art guidance for investigators who are preparing retrovirus vectors for clinical use. The motion was approved by a vote of 17 in favor, 0 opposed, and no abstentions.

XI. ADDITION TO THE POINTS TO CONSIDER OF THE NIH GUIDELINES REGARDING PROCEDURES FOR EXPEDITED REVIEW OF HUMAN GENE TRANSFER PROTOCOLS

Discussion

Dr. Walters summarized the deliberations of the Working Group on Expedited Review. The working group (Drs. Leventhal, Post, Zallen, and Walters) held a telephone conference call in order to develop the draft document entitled: *Procedures for Expedited Review*; the document was distributed to all of the members of the RAC for discussion. These *Procedures for Expedited Review* were originally proposed by Ms. Buc at the January 14 RAC meeting. The working group has incorporated several minor changes to the originally proposed procedures; however, these have not been substantive changes.

Dr. Krogstad reminded the RAC that he had originally suggested that protocols approved through the expedited review process should be formally reviewed at the next RAC meeting. Specifically, the expedited approval that was granted by the NIH Director in December 1992. This step would provide feedback from the RAC members to the individuals who are involved in the interim review process. This requirement does not appear in the draft document. There was a clear consensus of the RAC members on this issue. Dr. Leventhal agreed with Dr. Krogstad's comments and said that all of the RAC members should have access to the materials on which an expedited review approval is based.

Dr. Parkman said that the RAC would serve an important function by reviewing expedited approvals at the next regularly scheduled meeting. Approval or disapproval of an NIH-approved expedited review protocol would serve as a check and balance on the quality of the review process. Since the *Procedures for Expedited Review* were not incorporated as a major action to the *Points to Consider* of the *NIH Guidelines* at the time that the December approval was granted, the requirement for full RAC review at the next meeting could not be enforced.

Dr. Walters asked if the RAC intends to review expedited review protocols using the same standards of review as for other protocols. Dr. Parkman agreed that expedited review protocols should be reviewed by the same standards used for other protocols. Dr. Krogstad explained they would be evaluated for both scientific and medical merit. Dr. Wivel reminded the committee members that they had specified that investigators must also provide all of the materials that were submitted to the FDA in order to obtain approval.

Dr. Chase said that review of any protocol that receives expedited approval by the NIH Director is important information with regard to the rights of these patients. There is a chance that a decision for approval of an expedited review protocol by the Director could be contradicted by the RAC. This information would be invaluable to those individuals who might be tempted to believe that any treatment approved by the government is considered scientifically sound and efficacious.

Dr. Leventhal said that the RAC should not refer to expedited approval as compassionate plea exemptions. The term compassionate indicates that there is some inherent value to the procedure. Expedited approvals may have no experimental value; therefore, they should not be considered compassionate. She cautioned the committee to consider the thousands of terminal cancer patients who will hear about this expedited approval process and have their hopes raised that they may also qualify for such an exemption. Dr. Parkman said that the *Procedures for Expedited Review* includes the provision that expedited approval will be granted only for a single patient; this provision should obviate any concerns about raising false hope for terminal patients. Dr. Walters concurred with the statement made by Dr. Parkman and emphasized that the onus is on the investigator to provide a valid argument for why the patient cannot wait until the next scheduled RAC meeting. The expedited review process is not viewed as a mechanism for accruing multiple patients onto a single protocol.

Dr. Leventhal reminded that committee that Dr. Wilson is an example of an investigator who identified several patients who did not fit the inclusion criteria for RAC approval. However, Dr. Wilson had sufficient time to request a minor modification to his approved protocol and to present the unique set of circumstances to the RAC at its regularly scheduled meeting. Dr. Miller reminded the committee that Dr. Wilson's request was not for expedited review, but was submitted as a minor modification to a previously approved protocol.

Dr. Parkman said that the objective of the *Procedures for Expedited Review* is to ensure that the scientific quality of the protocol is as good, if not better, than the quality of those protocols approved through the regular review process.

Cover Sheet for Expedited Review

Dr. Parkman presented a draft checklist of critical information that should be completed at the time that an initial request is made by an investigator for expedited review of a single patient protocol. This *Cover Sheet for Expedited Review* will be used by the Office of Recombinant DNA Activities (ORDA) to determine whether the investigator has minimal requisite preclinical data which would warrant expedited review. If the requesting individual cannot complete the entire *Cover Sheet for Expedited Review* than their request will not be forwarded based on the fact that the requisite minimal data to evaluate the protocol does not exist. If the investigators do not have the vector sequence, gene transduction or expression data, or safety data, then it is not appropriate to evaluate the protocol.

Dr. Post expressed concern that this 1 page document may send a signal to investigators that they can submit less information for an expedited review protocol than for a protocol reviewed through the normal process. Investigators should be required to

submit the *Points to Consider*. Dr. Parkman said that the *Cover Sheet for Expedited Review* is not intended to outline all of the documentation that is required for submission; it is intended as a screening process.

Dr. Haselkorn stated his objection to the use of the word "therapy" in item number 4 of the *Cover Sheet for Expedited Review*. Dr. Wivel suggested that the word "transfer" be substituted for the word "therapy". Dr. Haselkorn agreed to this substitute wording. Dr. Haselkorn suggested that expedited review should only be considered for post-Phase I trials. Phase I studies are inappropriate for expedited review since there is no demonstrated therapeutic value.

Dr. Zallen suggested that the RAC should consider the public's perception and whether they will view these investigators as trying to take advantage of desperate individuals who can afford to pay for medical interventions. If the public develops this perception, the integrity of gene therapy research may be called into question. She suggested that a statement be included in the *Procedures for Expedited Review* requiring that all costs associated with the experimental protocol will not be borne by the patient or the patient's family. Inclusion of this criterion would maintain public confidence. Ms. Meyers agreed that the provision outlined by Dr. Zallen should be included in the final document. Dr. Leventhal reminded the RAC that this requirement would not preclude patients or their families from making monetary donations to an institution.

Dr. Chase stated that the RAC should remain confident that making this document "air-tight" will develop into long-term implications. One important factor that will be the peer pressure of responsible scientists in the area of gene therapy. Eventually, there may be an attempt to discredit those investigators who repeatedly make these types of requests. The other factor that will weigh heavily is the parallel recommendation by the RAC following NIH Director's determination. Eventually, the media and other individuals will influence public opinion. The public will realize that some of the experiments will have no medical or scientific value. It may take several years for the public to actually realize the implications of these expedited approvals. Dr. Krogstad recommended that item number 6 of the *Procedures for Expedited Review* include extramural scientists in the review of these protocols. In light of previous discussions about the possibility of political pressure being imposed on the review process, the inclusion of extramural scientists' review would avoid the appearance of such pressure being imposed.

Dr. Leventhal suggested that a question should be included in the *Cover Sheet* which requires that the investigators define the endpoint of the study, i.e., what will be measured? A clear example of the importance of endpoints is the patient who received expedited approval in December 1992. The RAC was told that the patient required emergency treatment because the tumor was growing rapidly. However, the RAC was

informed that the patient received hyperfractionated radiation prior to receiving the injections of gene-transduced cells. In addition, the patient's response was going to be measured compared to pre-radiotherapy. What would actually be measured in this situation? Dr. Parkman agreed that the experimental design must be valid in order accurately measure any potential response. Dr. Brinckerhoff said item number 1 of the *Procedures for Expedited Review* reads, "The NIH will strongly emphasize that the standard method of protocol submission is preferred." The words "strongly emphasize" are not forceful enough.

Dr. Anderson recommended that investigators should be required to indicate that the completed protocol, the *Points to Consider* document, and the vector sequence are attached as part of the completion of the *Cover Sheet for Expedited Review*. He said that this document should become a checklist of "yes or no" answers that can easily be completed by the requesting individual.

Dr. Leventhal suggested that the statement, "patient privacy will be maintained," should be included as part of item number 7 of the *Procedures for Expedited Review*. Also, the statement, "Protocols that are deferred or not approved by the RAC in its normal review process are not eligible for expedited review," will sometimes be difficult to determine. For example, Drs. Sobol and Royston had a protocol that was deferred. However, these investigators contended that the expedited review protocol submitted was a new protocol. In actuality, the differences between the 2 protocols are not very substantive. Investigators will always submit their request as a new protocol to satisfy this criterion.

Dr. Sobol asked about a scenario in which the investigator has data that would support an expedited review protocol. Dr. Leventhal said that the investigator should present the data at the next RAC meeting. Dr. Sobol asked about a situation in which the patient could not wait until the next meeting. Dr. Leventhal responded that the investigator would have to convince the RAC there are extenuating circumstances surrounding the experiment that are so specific that the opportunity will never present itself again to perform the experiment.

Dr. Sobol said that a situation could arise in which approval of a protocol is deferred by the RAC due to insufficient data, and the investigators obtain the requested data several weeks following the meeting. For example, under extraordinary circumstances a patient could meet the eligibility requirements of the deferred protocol, but the disease is progressing at such a rate that he/she was unable to wait until the protocol is reviewed at the next RAC meeting. Dr. Leventhal urged the RAC to keep this contingency as part of the *Procedures for Expedited Review*. The majority of protocols that are deferred by the RAC are missing significant data that cannot be obtained in a relatively short time frame. Protocols that are missing minor bits of data or information are normally approved with contingencies.

The members of the RAC continued to make minor modifications to the *Procedures for Expedited Review* and the *Cover Sheet for Expedited Review*. Dr. Walters suggested that a revised version of these two documents should be presented for final approval later on in the agenda.

Before presenting the next agenda item, Dr. Walters suggested that the RAC should form a working group to analyze and develop different categories for the classification of human gene transfer and therapy protocols. The purpose for this exercise is to assist the RAC in developing certain categories of protocols that may be eligible for decentralized review.

XII. ADDITION TO APPENDIX D OF THE NIH GUIDELINES REGARDING A HUMAN GENE THERAPY PROTOCOL ENTITLED: *A PHASE I STUDY OF GENE THERAPY OF CYSTIC FIBROSIS UTILIZING A REPLICATION DEFICIENT RECOMBINANT ADENOVIRUS VECTOR TO DELIVER THE HUMAN CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR cDNA TO THE AIRWAYS*/DRS. WILMOTT, WHITSETT, AND TRAPNELL

Review--Ms. Grossman

Dr. Walters called on Ms. Grossman to present her primary review of the protocol submitted by Drs. Robert W. Wilmott and Jeffrey Whitsett of Children's Hospital Medical Center, Cincinnati, Ohio, and Dr. Bruce Trapnell of GTI, Gaithersburg, Maryland.

Ms. Grossman provided a brief overview the proposed study. This protocol is a Phase I study to assess the safety and biological efficacy of a recombinant adenovirus delivered to the nose and lungs of patients with cystic fibrosis (CF). The investigators proposed to treat a total of 15 adult patients, who will be divided into 3 groups of 5 patients. Each group of patients will receive 1×10^{10} , 1×10^{11} , or 1×10^{12} plaque forming units (pfu) of recombinant adenovirus.

This protocol is more complex than the other CF protocols that have previously been reviewed by the RAC. This study involves the administration of vector to the nose and lung of the patients, followed by subsequent challenge with an equivalent dose of virus 2 months after the initial treatment. The proposed vector is an E1 and E3-deleted type 5 adenovirus. The cystic fibrosis transmembrane conductance regulator (CFTR) cDNA is expressed from a Rous sarcoma virus (RSV) promoter.

Ms. Grossman said that the investigators responded to the majority of her written comments; however, there is still one remaining question regarding the sequencing of the vector. The investigators indicate that they will confirm the sequence of the viral

backbone. They should describe the exact portions of the vector that will be sequenced. Also, the investigators need to present their quantitative PCR data demonstrating that they are able to detect 1 wild-type virus particle per 1×10^8 recombinant adenovirus particles.

Ms. Grossman said that there are several questions that the investigators should address during their oral presentation. What fraction of each dose will be evaluated for sterility, mycoplasma and replication-competent adenovirus? How will *in vivo* toxicity be determined? Toxicity studies have been performed in cotton rats; however, it would be preferable if these studies would be performed in a larger animal model. She stated a concern that the investigators are attempting to assess biological efficacy and safety of a single administration of recombinant adenovirus, and then they propose to rechallenge the patient with a second dose of virus 2 months later. The second administration of recombinant adenovirus may not be feasible and/or necessary. This second dosage may be putting patients at an additional risk since the effects of a single administration are unknown. She inquired whether recombinant adenovirus has ever been administered to a large animal model and an attempt made to obtain CFTR-expressing cells? Is there data demonstrating the investigator's ability to administer the recombinant adenovirus and recover gene-transduced cells from brushings?

Ms. Grossman noted that the inclusion/exclusion criteria limits eligibility for this protocol to patients greater than 18 years of age; however, the experiments will be performed at the Cincinnati Children's Hospital. Are there any necessary measures that must be taken to treat adults at a pediatric hospital? Who will be the primary physician?

Review--Dr. Haselkorn

Dr. Haselkorn stated that he was originally concerned about the necessity for frequent biopsies and the degree of discomfort associated with these procedures. The investigators did satisfactorily respond to these concerns by reducing the frequency of the bronchoscopies and biopsies.

Review--Dr. Zallen

Dr. Zallen said that she had the same initial concerns as Dr. Haselkorn regarding the frequency of biopsies, and that she was satisfied with the revised schedule. For clarification purposes, the investigators need to explain the necessity for the large number of x-rays and to explain their ability to reliably detect improvement in a lung that is already severely damaged.

Dr. Zallen stated that her written comments requested that a diagram be included to

assist the patient in understanding the sequence of events that they would undergo. A diagram has been submitted; however, the scale of the diagram is not proportional to time and should be revised. The investigators were commended for providing a comprehensive education program for health care workers that has been implemented for this study.

Other Comments

Dr. Parkman asked on what basis 56 days was chosen as the optimum time point for administration of the second dose of recombinant adenovirus. The reason that other investigators have proposed only a single administration is that the time frame for CFTR expression in humans is unknown. Although different adenovirus vectors will elicit varying degrees of immune response, Dr. Crystal has reported that the cotton rats have exhibited increased inflammatory responses for longer durations, in response to subsequent challenges of virus. What *in vivo* data exists with regard to the nature and duration of the inflammatory response with the proposed vector? Does the second challenge elicit a greater response than the first administration? Does the magnitude of the response increase with each consecutive administration?

Ms. Meyers noted that the informed consent document should include statements about long term follow-up and a request for autopsy. Dr. Walters asked the investigators to explain the role of GTI in the proposed study.

Presentation--Dr. Trapnell

Dr. Trapnell described the progress that has been made in the sequencing of the adenovirus vector. Restriction mapping has been performed to evaluate the structure of the virus. Sequence analysis has been initiated for both the plasmid and the backbone of the parental virus. GTI will sequence the entire vector; however, there is uncertainty about the necessity of sequencing the entire vector. If the RAC requires the entire sequence, GTI will comply with the request.

With regard to the fraction of the dose that will be evaluated for sterility, Dr. Trapnell stated that the exact fraction has not yet been determined because GTI is still in the process of scaling up the production volume. GTI's goal is to test an entire patient dosage. Ms. Grossman asked about the size of a production lot. Dr. Trapnell responded that a production lot will be equal to several patient doses; however, the exact number of doses has not yet been determined.

Dr. Miller asked if 1 helper virus particle can be detected in a 1×10^{12} vector particles, which is the maximum proposed dosage. Dr. Trapnell said that he would first like to summarize the possible methods for detecting replication-competent particles. The first

method is to evaluate the biologic titer of infected cells. The second method is to look for cytopathic effects and to evaluate for characteristic cell morphology which would indicate wild-type infection. The third method, which is more sensitive than the other 2 methods, is metabolic labeling of DNA in infected cells. The metabolic labeling method identifies the presence of wild-type genomic sequences, i.e., E1 sequences.

Dr. Trapnell presented data demonstrating metabolic labeling of genomic DNA sequences in bronchial epithelial cells that were obtained by bronchoscopy. Following DNA extraction, enzyme restriction analysis is performed using agarose gel electrophoreses and autoradiography. The wild-type and recombinant virus have distinguishable restriction fragment patterns. PCR analysis is being used to detect the presence of replication-competent adenovirus particles. The PCR method is several logs more sensitive than the other methods. A reconstruction experiment was presented in which wild-type virus was mixed with the recombinant virus at different ratios. The cells are heat denatured, and PCR amplification is performed with both the E1a and E2b primers. Next, these samples are subjected to Southern blot analysis. The PCR amplification method demonstrates that 1 wild-type virus particle can be detected in 1×10^9 vector particles. This assay sensitivity is the current level.

Dr. Miller said that patients will receive as much as 1,000 times the number of vector particles used in the reconstruction experiment. Dr. Parkman said that with this level of sensitivity, the investigators can only say that there is less than 1,000 replication-competent particles per patient dose. Dr. Trapnell said that plaque purification will be performed; therefore, the theoretical number of replication-competent particles will be much less than 1,000. Ms. Grossman inquired if there are other cell-based assays that could be performed to increase the level of sensitivity. Dr. Trapnell responded that the cytopathic effect assay could be performed; however, the necessary dilutions would require thousands of plates, which would make assay prohibitive.

Dr. Trapnell presented data from other trials in which wild-type adenovirus was delivered to the respiratory tract of a large number of individuals. This trial was conducted between 1953 and 1970. These individuals received as much as 4×10^5 wild-type particles. The most severe symptoms consisted of mild upper respiratory illness, i.e., coughing and rhinorrhea. These symptoms were consistent with those observed in patients with a typical adenovirus induced cold. For individuals who demonstrated preexisting neutralizing antibody from a prior infection, there was an inverse relationship between the amount of antibody and the development of asymptomatic infections. Patients enrolled in this study will have demonstrated neutralizing adenovirus antibody. There has never been any instance of malignancy associated with adenovirus infection in humans.

Dr. Motulsky inquired if these trials have been followed long-term. Dr. Trapnell said

that some of these studies have been studied long-term, and that 4×10^5 adenovirus particles is considered to be a safe dose. Dr. Post commented that this number of particles are safe to administer to a normal patient. What would the effect be in CF patients? Dr. Wilmott responded that CF patients are exposed to adenovirus particles at the same rate as normal individuals. In general, CF patients are hyperimmune and demonstrate high levels of gamma globulin, particularly IgG. There is no reason to believe that these patients will manifest any problems specifically associated with adenovirus infection.

Presentation--Dr. Whitsett

Dr. Whitsett responded to Ms. Grossman's concerns about *in vivo* toxicity. The cotton rat was chosen as the *in vivo* model because of its unique susceptibility to adenovirus. Data was presented in which cotton rats were given 3×10^{11} pfu intratracheally. Substantial gene expression was observed out to 7 days. These animals demonstrated lymphocyte and macrophage peribronchiolar and perivascular responses in response to vector administration. Recently, this same response has been observed in the hamster model. Dr. Whitsett explained that rabbit experiments are currently in progress; 3 animals have been treated to date. These 3 rabbits demonstrated responses similar to those observed in the cotton rats and hamsters. The animals did not become ill. They did not breath rapidly or have cyanosis. However, pathology demonstrated significant lymphocytic and monocytic infiltration by hematoxylin and eosin (H&E) staining. There was no evidence of pneumonia or hemorrhage.

Dr. Whitsett explained that the investigators are concerned about demonstrating the safety of multiple administrations, and that they will continue to perform repeat dose experiments in large animals. The human protocol will not be initiated until these large animal experiments have been completed and evaluated.

Dr. Whitsett explained that repeat administrations will be necessary because of the turnover of the respiratory epithelium. The time frame involved in cell turnover is between 80 and 100 days. Conditions will be optimized such that CFTR expression can be achieved throughout this entire period. The date chosen for the second administration, 56 days, is based on data which demonstrates that this period is where one-half of the original mRNA is detectable within the lung. All patients will have neutralizing antibodies to the adenovirus which should obviate any concern about an aggressive immune response to the vector.

Dr. Whitsett stated that he is compelled to proceed with this protocol as a feasibility study in terms of the repeat administration of the adenovirus vector. The entire strategy of CFTR transduction in the lung is dependent on multiple administration of the vector. Ms. Grossman said that patients should not be exposed to a second administration of the

vector if the effect of a single dose is unknown. There is a possibility that patients may not demonstrate a clinical response at the lower concentrations; therefore, why would you subject a non-responsive group of patients to a second administration? Dr. Whitsett explained that if the objective of the protocol was to demonstrate efficacy, then there would be a flaw in the experimental design. However, the objective of the protocol is to determine toxicity and whether a second dose of vector is feasible. This study is designed to determine safety, not efficacy.

Dr. Parkman said that it is difficult to approve a protocol in which a second administration of vector has not been tested in a large animal model. He said that he would more readily vote for approval of the protocol if it involved only a single administration of the vector. Dr. Whitsett stated that a single dose would be a fall-back position; however, it would be preferable to proceed with the protocol as it was originally proposed. Ms. Grossman said that she would prefer that only a single administration be approved by the RAC until such time that *in vivo* preclinical data was available in a large animal model. Dr. Whitsett requested permission to change the design of the protocol to include a single administration of the vector instead of 2 doses.

Dr. Miller inquired about the effect of neutralizing antibodies on the adenovirus vector. Dr. Trapnell stated that *in vivo* data demonstrates that adenovirus infection is possible in the presence of overwhelming levels of serum antibody. The lymphocytic and monocytic immune responses that are observed are transient regardless of whether animals are immune.

Dr. Trapnell responded to questions regarding the ability to detect gene expression. A technique was developed for evaluating gene expression in the respiratory epithelium using bronchoscopies to obtain brushing samples. Millions of ciliated and secretory cells can be obtained in this fashion. Gene expression is evaluated by quantitative PCR. Gene expression has been demonstrated in respiratory tract cells from the nose through the bronchus; however, gene expression is depressed in the pharynx. Comparison between quantitative PCR and the amount of hybridization will provide an estimate as to the copy number per cell.

Ms. Grossman asked the investigators to describe the *in situ* hybridization assays that have been performed. Dr. Whitsett explained that the investigators have been successful in distinguishing both the endogenous and transferred gene by *in situ* hybridization. Dr. Whitsett stated success at detecting several copies of CFTR per cell by this method.

In response to Dr. Zallen's concerns, Dr. Whitsett said that a revised flow chart has been prepared to assist the patient in understanding the various procedures and time points; however, the second administration phase will be deleted. Patients will be followed by Dr. Robert Baughman who is the Director of the adult program of the Cystic Fibrosis

Center.

Dr. Whitsett stated that all patients will be followed long-term, and that an autopsy will be requested in the event of death. With regard to the number of x-rays that will be required, the number of x-rays will be greatly reduced because the second dose of vector will now be deleted. As the protocol was originally written, the proposed dosage of radiation was within the National Radiation Safety Council's Guidelines. The new dosimetry will be half of what was outlined in the original protocol. With regard to the ability to measure potential differences (PD), data was presented demonstrating a nasal PD of 80 millivolts in a CF patient as compared to 10-20 millivolts in normal individuals.

Dr. Whitsett concluded that the investigators' intent is to formally remove the rechallenge portion of the protocol. The second dose protocol will be resubmitted to the RAC when the *in vivo* data is complete and the clinical efficacy of the initial trials has been evaluated.

Committee Motion

A motion was made by Dr. Haselkorn and seconded by Dr. Motulsky to approve the protocol as amended to include a single administration of the adenovirus vector.

Dr. Miller said that the RAC has always required that there be less than 1 adenovirus helper virus particle per patient dose; however, this requirement may be unnecessary. The problem is that there is no solid data with regard to the number of particles that will produce complications in a CF patient. Dr. Trapnell explained that CF patients are routinely exposed to adenovirus infections at the same rate as normal individuals, and both groups demonstrate similar clinical responses as demonstrated by gross examination.

Ms. Grossman asked if the patients enrolled into this protocol will have be sicker than the average CF patient. Dr. Wilmott responded that the eligibility criteria includes patients who demonstrate mild to moderate disease. Initial patient accrual will include patients with mild disease.

Dr. Parkman asked about the threshold dose to get a wild-type adenovirus infection. Dr. Whitsett answered that one-half of all individuals get a cold when exposed to concentrations of between 1×10^3 and 1×10^4 adenovirus particles. It is unknown how many adenovirus particles are transmitted in a human cough. Dr. Miller noted that 1×10^3 particles is not an enormous dose; therefore, it is reasonable to allow this upper limit.

Dr. Straus explained that the argument that CF patients have normal immunity to adenovirus is somewhat flawed, because all individuals acquire these infections at a

young age. Data has demonstrated that 100% of the population is exposed to adenovirus types 1, 2, 5, and 6 by the age of 3 years. This early exposure occurs at a time when the lungs are still relatively normal in CF patients. The protocol is designed to treat CF patients whose pulmonary function is no longer normal. Dr. Miller explained that advanced CF patients do not demonstrate clinical symptoms in response to adenovirus exposure. Dr. Straus acknowledged that these patients do not demonstrate bronchiolitis or pneumonia. However, these patients are being exposed to low titers and do demonstrate evidence of mucosal immunity. These patients have never had large quantities of adenovirus particles delivered into their respiratory tract. The capacity of these patients is still unknown; therefore, the RAC should not prematurely approve excessive levels of wild-type exposure.

Dr. Parkman said that the criteria for this protocol are similar to those standards established for the other CF protocols approved by the RAC. Dr. Miller said that the RAC should exercise consistency and ask investigators to demonstrate the ability to detect 1 wild-type particle per patient dose. Dr. Post said that there is reason to believe that this requirement is unnecessary. Dr. Miller suggested that he would recommend approval of the protocol with the stipulation that the investigators try to increase the level of sensitivity of their assay for the detection of wild-type adenovirus; however, the current standards are acceptable at this time.

Dr. Haselkorn suggested that perhaps the level of PCR sensitivity could be increased by the addition of biotinylated nucleotides in the late steps and using radiolabelled avidin to detect the product. Another option is to perform a reconstruction experiment with 1×10^{12} vector particles and identifying replication-competent virus following serial passage. Dr. Trapnell explained that one of the difficulties in identifying replication-competent particles is the number of cells that must be inoculated. This procedure could require as many as 2×10^4 plates to perform 1 assay. Even if this number of plates could technically be inoculated, it would be difficult to believe 1 plaque in 2×10^4 plates.

Dr. Trapnell said that it is reasonable to believe that the level of PCR sensitivity will be improved. However, even in the worst case scenario, these patients would not be exposed to levels of wild-type virus that are any higher than the natural situation. Dr. Post asked if assays have been performed with A549 cells. Dr. Trapnell said that assays have been performed with A549, HeLa, human embryonic kidney, and 293 cells. There are problems associated with each of these cells. These systems reach their capacity in terms of the number of vector particles per cell; therefore, dilutions are still necessary.

Dr. Parkman reminded the RAC that the limit of 1×10^3 is the worst case scenario of replication-competent particles; the actual number could be 0. Dr. Trapnell agreed with Dr. Parkman noting that the initial group of patients who will receive 1×10^9 vector particles will receive vector preparations that have been demonstrated to have less than

1 replication-competent particle. Dr. Haselkorn asked if the current PCR amplification utilizes a nested procedure with multiple rounds of amplification. Dr. Trapnell explained that the investigators are in the process of optimizing the nested procedure, and that this method should increase the level of sensitivity.

Dr. Leventhal suggested that if the number of infectious particles is a real concern that the response of the initial 3 groups of patients can be used to evaluate toxicity. A stopping rule for toxicity should be based on the criteria that have already been developed. This study will provide valuable data about the number of infectious particles a CF patient can tolerate. Dr. Parkman responded that a direct correlation cannot be made since the actual number of infectious particles cannot be quantitated. Dr. Leventhal said that the committee should be confident that the experimental design of this protocol will not produce any extreme adverse reactions such as anaphylaxis.

Dr. Parkman moved the question. The motion to approve the protocol is contingent on the following stipulation: (1) that the second administration of the adenovirus vector, Ad1CF2, and associate clinical procedures will be eliminated from the protocol and the informed consent document. The RAC recommends that the investigators attempt to obtain a level of sensitivity adequate to detect 1 replication-competent virus particle per patient dose. The protocol was approved by a vote of 16 in favor, 0 opposed, and 2 abstentions.

XIII. ADDITION TO APPENDIX D OF THE NIH GUIDELINES REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: *GENE TRANSFER FOR CYSTIC FIBROSIS USING E1 DELETED ADENOVIRUS: A PHASE I TRIAL IN THE NASAL CAVITY*/DRS. BOUCHER AND KNOWLES

Review--Dr. Post

Dr. Walters called on Dr. Post to present his primary review of the protocol submitted by Drs. Richard C. Boucher and Michael R. Knowles of the University of North Carolina, Chapel Hill, North Carolina.

Dr. Post explained that this protocol involves the intranasal administration of an adenovirus vector containing the gene coding for CFTR. The advantages of nasal versus lung administration is that the epithelium is easily accessible for biopsies and electrochemical analyses.

This protocol will use the same adenovirus vector that was approved for Dr. Wilson's CF protocol at the December 1992 RAC meeting. Dr. Wilson, a co-investigator on this study, and will supply the vector. This vector has a cytomegalovirus (CMV)-enhanced β -actin promoter with an SV40 polyadenylation signals inserted into the E1 region. One

important difference between this protocol and Dr. Welsh's nasal administration protocol is the proposed doses of vector. Dr. Welsh's protocol proposed doses between 2×10^6 and 5×10^7 pfu. In this protocol, patients will receive between 2×10^8 and 2×10^{11} pfu. Dr. Post added that similar doses of adenovirus vector have been approved for other CF protocols. The unique feature of this protocol is that the vector particles will be administered in a relatively small volume, i.e., 2 ml. The investigators propose that this concentration of virus will result in a high multiplicity of infection (MOI), i.e., 1×10^4 infectious viruses per cell.

Dr. Post explained that he was originally concerned that this high MOI might increase the probability of generating replication-competent adenovirus which could result in a cytopathic effect. The investigators have provided extensive discussion with regard to the *in vitro* versus *in vivo* effects of high MOIs. The investigators have proposed a rapid dose-escalation, i.e., a 30-fold increase in concentration per group. Dr. Post asked the other RAC members to state their opinions about the proposed doses. The investigators have provided supplemental data derived from extensive baboon studies. These studies involved a total of 15 baboons that received intranasal administration of 1×10^{11} virus particles. Analyses of these animals was still in progress at the time of the original submission; therefore, the investigators should provide an update on this *in vivo* data.

Dr. Post asked about the status of vector sequencing. The investigators' written responses indicated that additional sequencing information would be available at this meeting. The RAC should consider whether complete sequencing of adenovirus vectors is a requirement. The RAC has always required that an investigator submit the vector sequence, yet the committee has never provided specific details.

The investigators have proposed a very stringent assay for the detection of helper virus. In light of the discussion of Drs. Wilmott, Whitsett, and Trapnells' protocol, perhaps the assays proposed for this study are too stringent. What is the difference between helper virus assays #1 and #2? Which assay(s) will be performed? How is a determination made as to the cell types that are recovered by bronchial alveolar lavage (BAL)? Can respiratory epithelial cells be distinguished from infiltrating lymphocytes?

Dr. Post complimented the investigators for submitting a well-designed, readable, and thoroughly documented protocol. He stated that if the investigators provide satisfactory responses to his questions, he would recommend approval of the protocol.

Review--Dr. Motulsky

Dr. Motulsky stated that the protocol has been thoroughly documented, and that he is very satisfied with the overall design of the study. The investigators should respond to several issues during their presentation. What is the possibility that patients will produce

antibodies in response to the modified adenovirus? Is there any possibility of homologous recombination which would result in a pathogenic virus. Is there any concern about over-expression of the CFTR gene? Is it legal to confine patients for up to 3 days if they are excreting virus if they do not want to be confined? Data demonstrates that the gene does not penetrate the submucosal glands. How important are these glands at restoring complete function?

Review--Dr. Zallen

Dr. Zallen explained that her initial written comments were regarding the source of the vector and the use of the term "gene therapy" in the informed consent document. The proposed vector will be supplied by investigators who are moving from the University of Michigan to the University of Pennsylvania. She inquired whether this transition would create any unforeseen problems with regard to obtaining the vector. Also, the RAC should consider the appropriateness of the term "gene therapy" since this protocol is a Phase I study that has no therapeutic intent.

The statement, "I am unlikely to gain any medical benefit from this study" should be revised to a stronger statement such as, "I will not get any benefit from the study." It is unlikely that transduction of a small portion of the nasal epithelium with the CFTR gene will result in any benefit to the patient's disease.

The investigators' responses to the *Points to Consider* were not complete. The listing of the questions far exceeds the responses. The reader is prompted to refer to the protocol for most of the answers. The *Points to Consider* is a document that is read by a much larger audience than the RAC; therefore, the committee should encourage investigators to provide complete responses to the document. The RAC should begin to consider an issue that is not particular to this protocol. Specifically, the RAC should discuss whether a protocol that has been approved at one institution can be conducted at another institution. There may be a great deal of variability in the conditions between institutions. Dr. Walters agreed with Dr. Zallen that the RAC should begin discussion on this issue.

Other Comments

Ms. Meyers noted that the investigators have provided a model statement about a request for autopsy in the informed consent document. All investigators submitting human gene transfer protocols for review by the RAC should include a similar statement.

Dr. Chase agreed with Dr. Zallen's comment about the use of the term "gene therapy." Dr. Leventhal suggested that "gene transfer" is acceptable alternative wording. This language has been adopted for other protocols.

Dr. Straus stated that the investigators need to expand on the status of the baboon studies. Is it true that human adenoviruses do not replicate in baboon cells? Is the baboon a relevant animal model? Will individuals be excluded from participation in the study if they demonstrate expression of E1 gene products. This stipulation was part of the exclusion criteria for Dr. Crystal's protocol. E1 has been shown to complement adenoviruses. Dr. Parkman explained that during the course of reviewing Dr. Crystal's protocol, Dr. Harold Ginsberg (an *ad hoc* consultant during the December 1992 meeting) stated that complementation by E1 should not present any deleterious effects; therefore, this exclusion criterion is unnecessary.

Presentation--Dr. Boucher

Dr. Boucher explained that the nasal epithelium was chosen as the target for this Phase I study because it provides a defined region that can be easily assessed in order to obtain electrochemical measurements and biopsy samples. Valuable information will be obtained about the safety and efficacy of the gene transfer procedure. The respiratory epithelium of the nasal cavity is very similar to the airways; therefore, it is a very pertinent model.

He presented data demonstrating the fraction of cells in the epithelial sheet that have to be corrected in order to restore the ion transport defect that characterizes CF. Using an artificial system of immortalized epithelial cells, gene transduction correct the electrophysiological defect in approximately 10% of the epithelial sheet. This system is artificial, and it is unclear that the same results will be observed *in vivo*. A transduction rate higher than 10% will probably have to be achieved in humans to see any significant correction in the electrophysiological defect.

He presented *in vitro* data demonstrating that approximately 1×10^{10} pfu are required to obtain maximum gene transfer in both nasal and bronchial epithelial cells. Therefore, the nasal cavity should represent a good model in which to study the efficacy of gene transfer in the lower airways. Experiments were presented demonstrating that the vector is capable of correcting the chloride transport defect in primary cultures of cells obtained from CF patients.

Using a murine model, the investigators have been able to demonstrate that the *in vitro* dose-response observation correlates with the *in vivo* response. Both human and murine nasal epithelial cells are transduced at the same rate with the proposed vector. Therefore, the mouse is an appropriate *in vivo* model for efficacy studies. The *in vivo* murine model was characterized. A cylinder is inserted into the trachea which allows for the administration of a known concentration and volume of virus. This area can be isolated for a defined period of time and provides the opportunity to determine the efficacy of gene transfer in a defined surface area. Exposure to 1×10^{11} pfu per ml for

30 minutes results in diffuse gene transfer, i.e., approximately 20% of the cells were transduced. This data suggests that higher concentrations of vector will probably have to be achieved in order to realize a therapeutic effect.

Assuming that very high concentrations of vector will be required, what are the safety considerations? Three days following intratracheal administration of 1×10^{11} pfu, no cellular response is observed in the submucosal area. However, there was a mild mononuclear infiltrate that was observed on day 7 which diminished by day 14.

Dr. Boucher explained the baboon model. The nasal mucosa of these animals was slowly perfused, particularly the inferior turbinate. Excess material drains anteriorly; therefore, the material is not swallowed and the possibility of ectopic expression is minimized. Dose-response studies in the baboon indicate that concentrations in excess of 1×10^{11} pfu per ml will be required to obtain efficient transduction.

Dr. Haselkorn asked Dr. Boucher to address the issue of submucosal resistance in the mouse. Dr. Boucher explained that although these tissues are resistant to wild-type adenovirus infection, there are receptors that are expressed in the nasal epithelium. The issue of gene transfer may not be dependent on the virus going through an entire life-cycle. Dr. Boucher introduced Dr. Wilson to provide additional information about the primate model.

Presentation--Dr. Wilson

Dr. Wilson explained that the investigators have established the primate model to determine toxicity. A total of 12 baboons, 3 animals per group, received between 1×10^7 and 1×10^{10} pfu per ml into their lung on the contralateral side. At a concentration of 1×10^{10} pfu per ml, 90% of the distal airway and alveolar cells were transduced. The cells of the proximal airway demonstrated sporadic gene transduction. A clear dose-response was observed. The gross necropsies were normal at all doses except at 1×10^{10} pfu per ml. On day 21, this concentration of vector demonstrated hemorrhagic reaction in localized segments of the lung. Other than the development of this infiltrate at day 21 and a slight fluctuation in their blood gases, the animals were clinically normal. These baboons have also been analyzed for the recovery of virus by BAL and nasal swabs. No virus was recovered from any of these animals.

Dr. Wilson commented on the status of the safety testing of the proposed vector. At the December 1992 RAC meeting, Dr. Ginsberg suggested that indicator cells are the method of choice for detecting replication-competent virus. However, the problem with indicator cells is that HeLa cells cannot tolerate a high MOI due to cytopathic effects. The maximum tolerated dose on HeLa cells was 100 pfu per plate. Dr. Ginsberg suggested using A549 cells. Dr. Wilson said that the investigators anticipated testing a

maximum of 1×10^9 pfu per production lot. A maximum of 1×10^6 pfu per plate of A549 cells were added. This large volume of plates should be manageable.

With regard to the status of sequencing, Dr. Wilson stated that the vector has been sequenced from the LTR through the promoters, through CFTR, through the polyadenylation signal, 6.12 kilobases into E2, and 3 kilobases surrounding the E3 deletion. The LTR, CMV, β -actin, the polyadenylation signal, and the CFTR gene were intact and functional. However, the sequencing analysis revealed that there are 3 base-pair changes. Two of these changes are identical to the rat sequence. This observation underscores the importance of sequencing the coding region.

In an attempt to discover why these base-pair changes occurred, the vector sequence was cross-checked with the plasmid and found that these changes were present in the original plasmid. Despite these changes, this CFTR allele is totally functional. Currently, the investigators are reconstructing the plasmid with known sequences. The new recombinant will probably be available in approximately 4 weeks. This protocol will not proceed until the sequence of the coding region has been determined.

Dr. Miller asked how the investigators can be certain that the entire viral DNA sequence is acceptable. Dr. Wilson responded that the sequencing is being performed by a contractor in 6 different orientations. The sequencing gel has been reviewed, and the data are correct. It is unusual that 3 mutations would occur, 2 of which are homologous to another species. Dr. Miller asked if the sequence obtained by the contractor has been cross-checked with the published sequence. Dr. Wilson said that he learned about the base changes only recently and has not had the opportunity to cross-check with the published sequence.

Dr. Parkman said that the standard should be the sequence of the vector that is going to be administered to the patient. Dr. Miller explained that the retroviruses have never been sequenced, only the plasmids. Even at that, investigators generally assemble sequences from the published literature as opposed to sequencing the plasmids themselves. The data that Dr. Wilson has provided goes beyond any sequence data that has been required previously by the RAC.

Dr. Wilson explained that the investigators have monitored the presence of antibodies to E2a in a variety of species, including primates, and none have been detected. With regard to the issue of recombination, Dr. Wilson stated that even in the worst case scenario, reconstitution of an E3 deleted wild-type virus should not result in any serious adverse consequences. In response to the question about carcinogenesis, Dr. Wilson stated that although there are data which demonstrates tumorigenicity in nude mice with other serotypes, there is no evidence that type 5 adenoviruses are tumorigenic.

Dr. Motulsky inquired if there is any coordination between the different laboratories that are conducting gene transfer research for the treatment of CF. Dr. Wilson responded that through the CF Foundation, there is substantial networking. The CF Foundation supports scientific meetings for the exchange of current information and data in the area of gene therapy.

Dr. Post asked Dr. Trapnell if the CFTR cDNA has been sequenced for the GTI supplied adenovirus vector. Dr. Trapnell answered that the plasmid was sequenced and found to be different from the sequence published in *Science*. However, Dr. Lap-Chi Tsui's laboratory said that transcriptional errors had been found, and that the sequence published in *Science* was not entirely correct. The *Science* paper had transcriptional errors that have since been corrected. The sequence derived at by GTI is the same as the final sequence obtained by Dr. Tsui's laboratory.

Dr. Post asked Dr. Wilson if he compared his CFTR cDNA sequence to the sequence published in *Science*. Dr. Wilson explained that he compared the sequence to the one in GenBank. Dr. Parkman said that the CF Foundation should identify the correct sequence of CFTR cDNA. Dr. Wilson stated that he will cross-check the sequence against Dr. Tsui's corrected sequence.

Dr. Straus stated that the RAC should be cognizant of the duplication of trials. Not only are these trials expensive, but they require a minimum number of subjects per trial. Each trial encompasses a certain degree of risk. With approval of 5 similar trials, these risks are encumbered at 5 centers. Although this approach may be exciting, it may ultimately prove to be non-therapeutic. Therefore, the RAC should emphasize the importance of animal models in assessing the safety of vectors. Dr. Boucher noted that the proposed study is a safety trial.

In regard to the question of over-expression, Dr. Boucher explained that a number of studies had been conducted in which CFTR was over-expressed in human epithelial sheets that were polarized. Data demonstrates that there is a level of saturation in the apical membrane. No evidence of expression in the contralateral membrane has been observed. CFTR seems to target in appropriate amounts. Dr. Boucher introduced his collaborator Dr. Knowles to respond to questions about the informed consent document.

Presentation--Dr. Knowles

Dr. Knowles addressed the issue of holding patients in quarantine. The IRB, the Institutional Biosafety Committee (IBC), and local health officials are in agreement that patients should be confined for 3 days to protect the public welfare. This statement will be included to inform patients that they will not gain any direct benefit from this particular protocol.

Dr. Knowles said that he prefers to use the term "gene therapy" because the objective protocol is to correct the cellular function of the cells in the nose. However, the terminology can be changed if required by the RAC. Dr. Miller said that the term "gene transfer" should be used instead of "gene therapy", because "gene therapy" implies that the corrected gene will restore function in the entire body.

Dr. Knowles explained that the issue of patient follow-up is very controversial at his institution. The patients will be a young adult population. There is a high probability that they will move several times within their lifetime. Is it necessary that they return to the institution after a year? Ms. Meyers stated that it is incumbent on investigators to follow these patients for life. Ms. Meyers reminded the investigators that the patients may not necessarily have to return to the institution. The long-term follow-up clause implies that the investigator will maintain a registry and contact the patient periodically with regard to their clinical status. Dr. Parkman reminded the RAC that the *Points to Consider* clearly indicates that patients will be followed for life.

Ms. Meyers commented on the previous discussion regarding the approval of duplicate or similar protocols. In the past 3 years, approximately 90% of the protocols reviewed by the RAC have been for the treatment of cancer. There have been very few protocols designed for the treatment of genetic diseases. The investigators should be applauded for pursuing multiple trials.

Dr. Post asked if there was data about the particular cell types that are transduced. Dr. Wilson responded that the majority of cells that are transduced are epithelial cells; however, there may be low level transduction of alveolar macrophages.

Committee Motion

A motion was made by Dr. Post and seconded by Dr. Miller to approve the protocol contingent on the following: (1) The correct sequence of the CFTR cDNA must be determined prior to administration of the vector to patients and provide a should provide a report at the next RAC meeting regarding resolution of the CFTR cDNA sequence. (2) Replace the phrase "gene therapy" with "gene transfer" throughout the document. The motion was approved by a vote of 16 in favor, 0 opposed, and 1 abstention.

XIV. PROPOSED ADDITION TO APPENDIX D OF THE NIH GUIDELINES REGARDING A HUMAN GENE THERAPY PROTOCOL ENTITLED: *PHASE I STUDY OF TRANSFECTED CANCER CELLS EXPRESSING THE IL-2 GENE PRODUCT IN LIMITED STAGE SMALL-CELL LUNG CANCER/DR. CASSILETH*

Review--Dr. Miller

Dr. Walters called on Dr. Miller to present his primary review of the protocol submitted by Drs. Peter A. Cassileth and Eckhard Podack of the University of Miami/Silvester Cancer Center, Miami, Florida. The objective of this protocol is to enhance localized cytokine secretion in order to stimulate an immune response against cancer cells. Tumor cells from patients with limited stage small-cell lung carcinoma (SCLC) will be transfected with a bovine papilloma virus (BPV) expression vector producing IL-2. Tumor cells expressing IL-2 will be irradiated to reduce their replication potential and re-introduced into patients after chemotherapy-induced disease remission. Animal data generated by these investigators in the Lewis lung carcinoma model supports the concept that such manipulations can induce rejection of implanted IL-2-secreting tumor cells. The investigators have shown that they can grow SCLC cells from human patients, and that the cells produce relatively high levels of IL-2 after transfection with the BPV vector.

Dr. Miller pointed out that this protocol is the first study designed to use a potentially oncogenic BPV vector as a vaccine. Sixty-nine percent of the BPV genome, including all early genes but not the late capsid genes, is included in the vector. These sequences include BPV genes with transforming potential. There is a concern whether the BPV vector is indeed replication-defective and that it does not pose any biohazard or possibility of spreading to other parts of the patient's body or to the population at large. The investigators have reviewed the previous literature and concluded that because of its size and lack of capsid proteins, the BPV vector is replication-defective. In his written review, Dr. Miller asked whether the BPV vector provides improved expression levels of cytokines in the human target cells over the standard retrovirus vectors that would clearly justify its use in humans. This question was not satisfactorily answered by the investigators. Also, there is a lack of convincing evidence in the animal model that injection of irradiated IL-2-secreting mouse tumor cells can promote rejection of existing tumor cells.

Review--Dr. Secundy (presented by Dr. Dronamraju)

In Dr. Secundy's absence, Dr. Walters called on Dr. Dronamraju to summarize her written comments. Dr. Secundy's concern was primarily with the informed consent document as follows: (1) the potential benefit statement appears too strong; (2) the financial responsibility of the institution in the event of unanticipated side effects of the treatment continues to be problematic, and (3) the question of unpredictable adverse consequences related to recombination of the vector with the human genetic material. In the revised consent form, the investigators addressed these concerns and included a statement about liability for costs of medical care engendered by side effects of therapy.

Review--Dr. Dronamraju

Dr. Walters then asked Dr. Dronamraju to present his comments on the protocol. Three different types of questions were raised: (1) Are the investigators proposing to use IL-2 alone or a combination of IL-2 and IL-4 therapy? In the animal model, encouraging results were obtained with a combination of IL-2 plus IL-4. (2) What is the transfection rate in human cells and the dose necessary to elicit a CTL response? (3) How was the sample size of 12 patients determined.

Other Comments

Dr. Leventhal found this protocol to be extremely confusing. The investigators should address the number of tumor cells to be obtained, the end points of the study, and the evaluation of the tumor response in patients who already responded to chemotherapy. It is doubtful that any valuable information will be obtained from this study.

Dr. Straus stated that Dr. Douglas Lowy of the National Cancer Institute has demonstrated that the 69% BPV genome contained in the vector can transform cells *in vitro* because the early viral genes interfere with cell cycle regulation. It is probable that the BPV vector will alter growth properties of tumor cells as well as normal cells.

Ms. Meyers made several comments regarding the informed consent document including the financial responsibility of institutions. She requested a statement should be included about the fertility status of patients, both males and females, and a suggestion that contraception should be used during the experiment.

This issue prompted a lengthy debate regarding the inclusion/exclusion criteria based on the fertility status of men and women. Dr. Miller and Ms. Buc questioned the scientific basis of requiring birth control during the trial. Dr. Parkman mentioned that drug companies are very firm on this requirement out of concern of any untoward effect to the fetus. Ms. Buc pointed out that contraception clauses will prevent the exclusion of women from protocols. Dr. Anderson added that this safeguard is good for public perception. In conclusion, the RAC members agreed that both men and women should be allowed to participate in protocols; however, they would be encouraged to use appropriate birth control.

Presentation--Dr. Podack

Dr. Podack stated that this protocol is similar to others that have used lymphokines to increase the immunogenicity of tumors in order to achieve an immune response that will be beneficial to patients. This protocol is for the treatment of SCLC. This tumor is particularly vicious with a mean survival of about 15 months; therefore, the potential benefit of this treatment outweighs the risk of the disease itself.

One major difference between this study and other protocols approved by the RAC is that this protocol is the first to utilize a BPV vector. In responding to a question from Dr. Miller regarding the infectivity of BPV, Dr. Podack stated that the 69% fragment of the BPV genome contained in the vector deletes the genes for proteins that are necessary to make an infectious particle. BPV virus is very difficult to grow *in vitro*, based on data reported in the literature over the past 15 to 20 years. The only way to obtain BPV is to extract the virus from a cow wart. Other reports have documented the absence of BPV sequences in slaughter house butchers who have repeatedly been exposed to bovine warts. In responding to the question about recombination of the vector with human papilloma viruses, Dr. Podack stated that based on extensive literature, no recombination of approximately 60 subtypes of human viruses has been reported. Papilloma viruses are very species-specific, a bovine virus will infect a cow, but not other animals. Responding to the question of transforming potential, Dr. Podack agreed that this risk is real. There are 3 potentially transforming genes, E5, E6 and E7, in these viruses. E5 has been shown to be transforming in both bovine and human species.

Discussion

In the present protocol, tumor cells are transfected with the BPV vector. Do these tumor cells become more aggressive? Dr. Podack stated that in SCLC cells, no morphology changes or changes in the rate of cell division have been observed. Dr. Miller asked whether the critical experiment has been performed in animals, i.e., have transfected and untransfected cells been injected into the Lewis lung carcinoma model. Dr. Podack answered that no differences in tumorigenicity have been observed in animal survival. According to literature, it is very difficult to demonstrate transformation in tissue culture with this vector. No transformed foci were observed in cells selected for G418 resistance. However, without selection, foci developed after long periods of time. The vector has not been conclusively demonstrated to be transforming. Dr. Nava Sarver of the National Institute of Allergy and Infectious Diseases, NIH, stated that she has shown that papilloma virus transformation can be demonstrated with the C127 cells both *in vitro* and *in vivo* in mice.

Dr. Miller commented on a question raised by Dr. Carmen that a radiation dose of 12,000 rads appears to be insufficient to kill all transfected cells. Dr. Miller was concerned that a new type of cancer might arise from the residual surviving cells. After reviewing Dr. Podack's data on cell survival following several doses of irradiation treatments, Drs. Smith and Miller agreed that 12,000 rads of irradiation does not kill all the tumor cells. Dr. Parkman stated that the radiation dose is already very high. Increasing the dose might compromise the transfected cells' ability to produce IL-2. A clonogenic assay would be a preferable method for determining tumor growth after radiation. Dr. Podack stated that he would conduct that assay if it is required for

approval.

Further questions were raised about *in vitro* studies that were performed for other purposes, with 4 tumor cell lines previously established by Dr. Niramol Savaraj, a co-investigator. Data on IL-2 production are not complete due to limited life span of these cells which already have been in tissue culture for several months. In the future, Dr. Savaraj will be responsible for establishing tumor cell lines from biopsies for the gene therapy protocol.

Dr. Dronamraju asked about Dr. Podack's contribution to a paper submitted with this protocol regarding combination effect of vaccination with IL-2 and IL-4 complementary DNA transfected cells and the induction of a therapeutic immune response against Lewis lung carcinoma cells. Dr. Podack stated that this animal model work was initiated while he visited the Japanese laboratory at the National Cancer Institute in Japan and was performed largely by scientists in that laboratory. In response to Dr. Dronamraju's question about IL-2 and IL-4, Dr. Podack stated that if IL-2 experiment is successful, then the combination of IL-2 and IL-4 will be used.

Dr. Parkman summarized the discussion noting that interesting pre-clinical data have been provided, but the protocol does not have all the elements to justify approval. Dr. Leventhal criticized the protocol for lack of any *in vitro* human data showing that transduced peripheral blood cells from tumor bearing patients demonstrate an enhanced anti-tumor response to autologous tumor cells from those patients. Dr. Parkman concurred that additional data should be provided using freshly established cell lines from tumor biopsies. Drs. Leventhal and Miller requested that an endpoint should be included, i.e., CTL assays. Dr. Podack said these studies will be performed in the future.

Committee Motion

A motion was made by Dr. Miller and seconded by Dr. Parkman to defer approval of the protocol. Approval is deferred until the investigators return to the RAC with the following: (1) a definition of the clinical endpoints, (2) clonogenic assays using irradiated tumor cells, and (3) a revised informed consent document, including a statement that some patients selected for gene therapy will require a second surgical procedure to obtain material for the study. The motion to defer approval of the protocol passed by a vote of 18 in favor, 0 opposed, and no abstentions.

XV. PROPOSED ADDITION TO APPENDIX D OF THE NIH GUIDELINES REGARDING A HUMAN GENE THERAPY PROTOCOL ENTITLED: *USE OF SAFETY-MODIFIED RETROVIRUSES TO INTRODUCE CHEMOTHERAPY RESISTANCE SEQUENCES INTO NORMAL HEMATOPOIETIC STEM CELLS FOR CHEMOPROTECTION DURING THE THERAPY OF OVARIAN CANCER/DR. DEISSEROTH*

Review--Dr. Leventhal

Dr. Walters called on Dr. Leventhal to present her primary review of the protocol submitted by Dr. Albert B. Deisseroth of the University of Texas M. D. Anderson Cancer Center, Houston, Texas. The objectives of this Phase I study are: (1) to test the feasibility of introducing the MDR-1 cDNA into normal hematopoietic early progenitor cells in advanced ovarian cancer patients by *in vitro* transduction with a retrovirus vector, and (2) to determine the effect on hematopoietic function following ABM transplantation.

Dr. Leventhal explained that the present protocol is similar to other studies reviewed by the RAC. The investigators will attempt to introduce the MDR-1 gene into ABM cells prior to transplantation so that higher doses of Taxol can be tolerated by the patient. Ovarian cancer rarely metastasizes to bone marrow thus lessening the chance that drug-resistant tumor cells will be introduced into bone marrow. There are several concerns that should be addressed regarding this protocol. *In vivo* murine data demonstrates that mice which receive transduced marrow cells recover more rapidly than those that receive untransduced cells. Dr. Leventhal asked if *in vitro* assays have been performed using human bone marrow cells. What is the transduction rate? What is the level of MDR-1 gene expression? The investigators need to provide a more detailed description of the vector, packaging cell line, and the reproducibility of experiments using the CD34(+) stem cells. The procedure for harvesting nucleated ABM cells should be described. Can 7×10^5 CD34(+) cells be reproducibly selected from 2×10^8 nucleated cells/kg? The protocol states that a total of 4×10^8 nucleated cells/kg will be harvested so that one-half of the cells can be cryopreserved as a back-up. Will harvesting this number of cells place patients at increased risk without blood transfusion? What is the relationship between G-CSF and Taxol administration? Is there any risk of toxicity in patients who have received prior anthracycline therapy with a daily dose of Cytosan? What is the end point of the protocol?

Review--Dr. Dronamraju

Dr. Dronamraju expressed concern about the informed consent document. The document does not clearly state that there will be no direct benefit to the patient, however, useful information will be obtained for the treatment of ovarian cancer in the future. The document should contain a request for autopsy.

Other Comments

Dr. Parkman asked about the parameters that will be used to define recovery of hematopoietic function following each administration of Taxol. Will the proposed dose of Taxol be tolerated by patients following ABM transplantation? Is Taxol a standard

therapy at M. D. Anderson Cancer Center for ovarian patients who have received ABM transplantation?

Ms. Meyers said that the informed consent document is not understandable to the lay person.

Presentation--Dr. Deisseroth

Dr. Deisseroth responded to questions raised by Drs. Leventhal and Parkman regarding Taxol administration in combination with ABM transplantation. Dr. Deisseroth stated that Taxol is not a standard therapy for ovarian cancer. Data demonstrates that Taxol can be administered to only 50% of all individuals because of myelosuppressive effects. The rationale for this protocol is that transduced hematopoietic cells with MDR-1 will increase the patient's tolerance to Taxol. With regard to the safety of Taxol administration in combination with ABM transplantation, Drs. Deisseroth and Kavanaugh explained they have had experience with patients who have received Taxol following an ABM transplant. Back-up marrow will be stored in the event that patients exhibit unusual sensitivity to Taxol.

Dr. Deisseroth presented *in vitro* human data that was not previously submitted. Taxol is routinely used to treat patients with advanced cancer. Unfortunately, therapeutic doses have been shown to induce severe leukopenia. At 250 mg/m², 67% of patients have demonstrated severe neutropenia. Myelosuppression is the major obstacle to the use of Taxol in ovarian cancer. The goal of this study is to assess whether introduction of the MDR gene into hematopoietic progenitor cells will decrease Taxol sensitivity. Ovarian cancer was chosen as the target disease because bone marrow involvement is rare.

The MDR cDNA contained in the vector (obtained by Dr. Michael Gottesman of the National Cancer Institute) has a point mutation which does not affect its function on drug efflux. The vector is derived from the LN vector series.

In response to Dr. Leventhal's question, Dr. Deisseroth stated that the second bone marrow harvest is necessary. Dr. Deisseroth stated that they have successfully established a procedure for selecting CD34(+) cells using a monoclonal antibody column. Following MDR transduction, the bone marrow cells were cryopreserved. The threshold for hematopoietic recovery following the preparative regime is 100,000 platelets per cubic microliter and an absolute neutrophil count of 2,000. Escalating doses of Taxol will be administered every 3 to 4 weeks in combination with MDR-1 transduced ABM cells. Is there a stopping rule for the trial, at doses 150 mg/m² of Taxol? GM-CSF is necessary to stimulate recovery.

FACS analysis will be used to monitor CD34(+) cells following transplantation.

CD34(+) cells will be grown in Dexter culture. Transduced MDR sequences can be distinguished from PCR endogenous sequences by this method. MDR-resistant cells will be enriched following each consecutive cycle of Taxol. A correlation will be made between the number of MDR transduced cells and the level of chemotherapy-induced neutropenia. Patients will be monitored following each dose of Taxol.

Dr. Deisseroth presented *in vivo* murine data. Data demonstrated that transduced bone marrow cells conferred a protective effect against Taxol. Dr. Miller noted that the vector used in the animal model is different from the vector proposed for the human study. Dr. Deisseroth acknowledged that the 2 vectors are different. Another objection made by Dr. Miller is that 5FU, which was used to enrich stem cells in the animal study, will not be used for human studies, a significant difference. Dr. Deisseroth presented preliminary data showing a protective effect of the vector that will be used in the human trial in the murine model. Drs. Miller, Leventhal, and Parkman all commented that this data should have been submitted with the protocol.

Dr. Post commented that there was no quality control data on the packaging cell line. Dr. Parkman expressed his concern that the potential risk to patients has not been adequately stated in the informed consent document, i.e., the risks associated with Taxol administration alone and in combination with transduced and untransduced ABM cells. If patients suffer from neutropenia, they may require transplantation of the cryopreserved marrow and a long costly hospital stay. Dr. Deisseroth conceded that the protocol may have to be revised incorporating a lower dose of Taxol in order to reduce the risk to patients.

Committee Motion

A motion was made by Dr. Leventhal and seconded by Dr. Parkman to defer approval of the protocol. Approval is deferred until the investigators return to the RAC with the following: (1) additional preclinical data on the infusion of CD34(+) ABM cells, (2) data demonstrating safety of the starting dose of Taxol, (3) a clear description of the proposed vector, (4) a revised informed consent document using simplified language and including a statement about the potential risks associated with Taxol administration following ABM transplantation (5) a revised title reflecting the fact that early progenitor cells will be used rather than stem cells, and (6) provide long-term cell culture data. The motion to defer approval of the protocol passed by a vote of 16 in favor, 0 opposed and no abstentions.

VI. ADDITION TO THE POINTS TO CONSIDER OF THE NIH GUIDELINES REGARDING PROCEDURES FOR EXPEDITED REVIEW OF HUMAN GENE TRANSFER PROTOCOLS (CONTINUED)

Discussion

The members of the RAC continued their discussion regarding the *Procedures for Expedited Review*. Dr. Walters summarized the changes that had been incorporated into the document based on the RAC's previous discussion.

A lengthy discussion ensued regarding limitation of expedited review to single patient protocols. The intent of this requirement is to define the limits for expedited review. The committee was concerned that investigators might interpret this statement as meaning that any single patient protocol will be reviewed as an expedited review protocol. The fact that a protocol involves 1 patient is not justification for expedited review.

The RAC discussed the appropriateness of using the phrase "gene transfer" for an expedited review protocol since the word "transfer" infers marking and the word "therapy" indicates an attempt to provide a therapeutic treatment. The members of the committee agreed that the term "transfer" should be used with the understanding that these are unproven Phase I trials, not marking studies. For clarification, the RAC added an additional item which specifies that the investigator must provide detailed information regarding the necessity of the expedited review.

The RAC debated whether the statement "Protocols that are deferred or not approved by the RAC in its normal review process, are not eligible for expedited review" should be deleted from the document. Since the committee was unable to come to a consensus with regard to this statement, a vote was called. A motion was made by Ms. Meyers and seconded by Dr. Parkman to include this statement as part of the *Procedures for Expedited Review*. The motion to retain this statement was approved by a vote of 12 in favor, 4 opposed, and no abstentions.

The committee agreed that extramural experts must be included in the expedited review process. The term "scientist" was changed to "expert" to reflect the importance of social and ethical review as well as scientific review.

Committee Motion

A motion was made by Dr. Chase and seconded by Dr. Miller to accept the *Procedures for Expedited Review* incorporating the minor modifications agreed upon by the RAC. The motion was approved by a vote of 16 in favor, 0 opposed, and no abstentions.

The revised version² of the *Procedures for Expedited Review* reads as follows:

Procedures to be Followed for Expedited Review

1. An investigator submitting a request to the NIH for expedited review of a gene transfer protocol must provide detailed information regarding the necessity of expedited review.
2. No protocol shall be considered without Institutional Biosafety Committee (IBC) and Institutional Review Board (IRB) approval.
3. At this time, all gene transfer protocols must be considered experimental.
4. Regardless of the method of review, the *Points to Consider* must be the standard of review for all gene transfer protocols.
5. Review of such protocols may include intramural NIH experts but must include extramural experts.
6. Among other factors to be considered by the reviewers, is the similarity of the new protocol to previously approved protocols.
7. The NIH will report to the RAC following expedited review and will include all of the materials on which the decision was based. The RAC will formally review the protocol at its next scheduled meeting. Patient privacy will be maintained.
8. Protocols that are deferred or not approved by the RAC in its normal review process are not eligible for expedited review. No protocol shall have more than one patient approved under expedited review.
9. As requested in the context of non-expedited review, none of the costs of the experimental protocol should be borne by the patient or the patient's family.
10. Data on all patients undergoing gene transfer shall be provided to the RAC within six months of the procedure.

Discussion

²Executive Secretary Note: The *Process to be Followed for Expedited Review* printed here is the approved NIH version. The NIH Legal Counsel revised item #9 to read "should" instead of "will." These procedures were published in the *Federal Register* on April 23, 1993 (58 FR 21741).

The RAC resumed discussion of the revised version of the *Cover Sheet for Expedited Review (Cover Sheet)*. This working document will be used solely by ORDA as a checklist for the minimum amount of information that is required for submission of an expedited review protocol for RAC review. Unlike the *Procedures for Expedited Review*, the *Cover Sheet* will not be incorporated into the *Points to Consider*. Several minor modifications were made to the *Cover Sheet* and agreed upon by the members of the RAC.

Dr. Royston questioned the intent of the RAC. He proposed the following scenario: If an investigator was approaching the deadline required for *Federal Register* notice, and the protocol is not in final form, would the request be deferred by the RAC if submitted in the incomplete format? Presuming that the protocol would be deferred due to insufficient information, should an investigator withhold submission of the protocol from the regular review process for submission through the expedited review process? Drs. Leventhal and Parkman stated that an incomplete protocol would not be accepted for either the normal or expedited review processes. All protocols, whether regular approval or expedited review, need to be complete and contain all of the information necessary to make a rational decision.

Committee Motion

A motion was made by Dr. Carmen and seconded by Dr. Miller to accept the *Cover Sheet for Expedited Review* with the incorporation of minor modifications agreed upon by the RAC. The motion passed by a vote of 15 in favor, 1 opposed, and no abstentions.

The revised version of the *Cover Sheet for Expedited Review* reads as follows:

COVER SHEET FOR EXPEDITED REVIEW OF A GENE TRANSFER PROTOCOL

1. What is the gene and vector proposed for the gene transfer protocol?
2.
 - a. Has the vector been previously approved by the RAC?
 - b. If not, attach complete sequence of vector.
3. What is the target cell for the gene transfer?
4. What is the transduction efficiency of the vector into the proposed target cell?
5.
 - a. What is the level of expression in the target cell?
 - b. How was expression determined?

6. What system was used to determine pre-clinical efficacy?
7. What is the endpoint of the protocol?
8.
 - a. What assay was used to detect replication competent helper virus?
 - b. Was replication competent helper virus detected? Yes ___ No ___
 - c. What is the level of sensitivity of the assay? (Attach documentation)
9.
 - a. What was the date of IRB approval? _____ (Attach copy of approval)
 - b. What was the date of IBC approval? _____ (Attach copy of approval)
 - c. What was the date of IND submission to the FDA? _____
10.
 - a. If the FDA permits the protocol to proceed, is clinical grade material available? Yes ___ No ___
 - b. If clinical grade material is not available, on what date will it be available?

XVII. DISCUSSION REGARDING THE HUMAN GENE TRANSFER PROTOCOL MATERIALS SUBMITTED FOR APPROVAL ON A COMPASSIONATE USE BASIS/Drs. Royston and Sobol

Background

In a letter dated December 7, 1992, Dr. Ivor Royston of the San Diego Regional Cancer Center, San Diego, California, requested compassionate plea approval for a human gene transfer protocol. The protocol was entitled: *Phase I Study of Cytokine Therapy of Cancer, Active Immunotherapy of Glioblastoma with Tumor Cells or Fibroblasts Genetically Modified to Secrete IL-2*. On December 28, 1992, the NIH Director and the FDA granted approval of Dr. Royston's request on a compassionate use basis.

On January 14, 1993, the NIH Director called a special meeting of the RAC to discuss expedited review procedures. During this meeting, Dr. Royston stated that he would provide the RAC with additional data that was supplied to the FDA. Dr. Royston said that the FDA-submitted material included the following: (1) additional safety data on

the transduced cell line, (2) sterility data, (3) safety data on replication-competent virus, (4) vector identity data, and (5) data on the level of IL-2 production. The RAC requested that documentation submitted to the IRB and IBC should be included.

Discussion

Dr. Post asked Dr. Sobol if the materials submitted to the RAC are the same as those submitted to the FDA? Dr. Sobol responded that it was his understanding that the general information provided to the FDA should be submitted. Dr. Sobol said that the most of their discussions with the FDA were by way of telephone communication. Dr. Sobol stated that the document submitted to the RAC is not an exact duplicate of the FDA submission. Dr. Sobol stated that the FDA was supplied with a more detailed description of the protocol, procedures, assays, etc. Dr. Sobol said that the information provided to the RAC is a summary of the information that the FDA had at the time that compassionate approval was granted.

Dr. Chase stated that the RAC never received the *Points to Consider* for this protocol. At the January 14 meeting, Dr. Sobol stated that the *Points to Consider* would be submitted in addition to the FDA-submission for review at the March RAC meeting. Dr. Royston stated at the January 14 meeting that the *Points to Consider* had been prepared and were back at his office. Dr. Wivel explained that the transcripts reflect the fact that Dr. Royston offered to submit the *Points to Consider*; however, the formal request was only for the FDA submission.

Dr. Parkman asked if data had been provided to the FDA regarding the level of IL-2 expression by either transduced tumor cells or fibroblasts. Dr. Sobol said that this data was submitted to the FDA. Dr. Miller stated that the information provided to the RAC is deficient in several areas; the protocol would never have been approved by this committee. Dr. Miller asked if data was provided to the FDA about the effectiveness of irradiation in inhibiting growth of the injected tumor cells. Dr. Sobol stated that this information had been supplied to the FDA. Dr. Sobol apologized for omitting this data from the RAC submission, and that this omission was an oversight.

Dr. Miller asked the investigators if they submitted any *in vivo* efficacy data to the FDA. Dr. Royston answered that no IL-2 efficacy data in a glioblastoma model was provided. The only efficacy data was the *in vitro* colorectal carcinoma data. Dr. Miller asked how they determined the number of IL-2-secreting cells used for injection if the level of IL-2 expression *in vivo* is unknown. Dr. Royston said that the number of cells injection was based on information derived from literature and data derived from the *in vitro* colorectal carcinoma model.

Dr. Royston said that this request was supported by many oncologists and scientists of

the National Cancer Institute who were of the opinion that efficacy data is unnecessary. Dr. Miller said that the RAC does not necessarily agree about the requirement for efficacy data. Dr. Royston stated that if the RAC had not approved this protocol then he would have been forced to challenge the review system.

Dr. Leventhal stated that the protocol approved by the NIH Director and the FDA is not what would normally be considered an emergency protocol. Dr. Leventhal stated that if a protocol is planned for only one patient, then it should be designed to answer a particular question. This protocol has no endpoint. Dr. Royston said that the *Procedures for Expedited Review*, just approved by the RAC, do not include long-term plans. Dr. Leventhal reminded Dr. Royston that this document does include the statement that the single patient protocols must be consistent with the *Points to Consider* which encompasses long-term planning.

Dr. Straus expressed concern that the protocol followed by the investigators is different than the protocol submitted to the FDA. Dr. Sobol said that if the RAC perceives that there was a deviation from the protocol, the error is probably that the statements were not worded clearly enough. Dr. Sobol said that in his opinion the treatments administered to the patient were articulated in the protocol.

Dr. Chase said that the key issue is that the RAC does not have enough information to determine whether it would have voted to recommend approval of this protocol on scientific grounds at the time that the NIH Director approved the request. One of the important aspects of the RAC is the degree of confidence that the public has in the decisions made by this committee. The public deserves to know that the RAC has made an informed decision about the value of an experiment. A decision made by the RAC about a research proposal may be different from a clinical decision made by a physician. As a physician, Dr. Healy granted approval of this protocol based on compassion for the patient. However, from a public standpoint, it is important that this protocol be evaluated for scientific merit by a panel of experts.

Dr. Royston stated that the public is primarily concerned about safety. Dr. Chase agreed that the public is concerned about issues of safety; however, there are other concerns. Dr. Royston said that the FDA looks at safety as well as the scientific merit of a protocol. However, the criteria used by the FDA may be different from those of the RAC. Dr. Chase said that one of the issues that the RAC is concerned about is the possibility Drs. Sobol and Royston might return to the NIH Director with other single patient expedited review requests, and that the data generated would not be interpretable by the scientific community. Dr. Chase stressed the importance that all investigators complete the *Points to Consider*. Dr. Chase stated that it is highly probable that if Drs. Sobol and Royston had completed the *Cover Sheet for Expedited Review*, the protocol would not have qualified for expedited review.

Dr. Royston stated that if the RAC retrospectively disapproved the protocol, there would be a greater understanding as to the perceived defects of the study. The RAC has already articulated their expectation that efficacy should be demonstrated in an animal model. There is disagreement about this particular requirement in the medical community. Also, there is disagreement about the extent of preclinical data that is required for a single patient expedited review protocol. Ms. Meyers reminded Dr. Royston that the RAC is not voting on this protocol; approval has already been granted by the NIH Director. Dr. Royston encouraged the RAC to retrospectively vote on this protocol to determine whether the committee's decision would have been the same as the NIH Director's decision. Ms. Grossman reminded Dr. Royston that the documentation submitted to the RAC is incomplete; therefore, it is impossible for the committee to make an informed decision. The purpose of this discussion is for the RAC, not the investigators.

Dr. Sobol stated that he would be unable to satisfy all of the criteria listed on the *Cover Sheet for Expedited Review*, even with the data that is currently available. There is no preclinical efficacy data, only historical data. Would the RAC have considered historical data in human subjects who were immunized with glioblastoma cells as an acceptable alternative to preclinical efficacy data? This proposal was based on data from a single uncontrolled study suggesting a survival benefit to patients who received adoptive immunotherapy of glioblastoma cells. The rationale is that gene transfer to these adoptively transferred tumor cells should enhance the survival benefit. There is no evidence that these procedures should produce any toxic side effects.

Dr. Parkman said that the fact that 1 cytokine gene has proven effective in the regression of 1 particular type of tumor does not establish preclinical efficacy for another cytokine or another tumor type. Dr. Sobol disagreed with Dr. Parkman's statement. Dr. Post noted that the uncontrolled study referenced by Dr. Sobol was reported a long time ago. Have any controlled follow-up studies been published since the original report? Dr. Sobol said that subsequent trials have been performed in which efficacy was not demonstrated.

Dr. Straus said that the urgency of illness sometimes necessitates that clinicians extend beyond the reach of existing scientific data. There are circumstances in which protocols could be justified without having demonstrated efficacy in an animal model. However, Phase I trials of this nature usually include therapies for which there is extensive data about the characteristics and safety of these therapies. Gene therapy is a novel therapeutic approach. Until such time that there is confidence in this area of research, it would be inappropriate to presume that protocols should be approved that go beyond the boundary of existing data. Dr. Sobol disagreed with Dr. Straus. Dr. Sobol stated that he does not see any distinction between what is considered acceptable for chemotherapeutic agents versus gene transfer.

Dr. Carmen asked the investigators how they would have responded to the question about transduction efficiency as listed on the *Cover Sheet for Expedited Review*. Dr. Sobol responded that the percentage of cells transduced is not an important issue; the key factor is the amount of IL-2 that is expressed. Ms. Grossman said that if an investigator cannot demonstrate reliable and efficient transduction of cells, then the protocol should not be approved. Dr. Royston asked, "If we have a selected cell line that is transduced and secreting the gene product, what is the concern?" Ms. Grossman said that the basic issue is that the RAC must be certain that investigators are capable of transducing genes into target cells. Dr. Sobol agreed that gene expression by the target cell must be demonstrated; however, the efficiency of the transduction should not be a decisive factor.

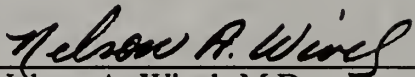
Dr. Sobol said that the first time any agent is administered to a human subject, the effects of a particular dosage are unknown. Only by monitoring the patient's response can the appropriate dose can be defined. Deferring approval of a study in which the only way to obtain data is in the human subject suggests that the RAC is too focused on details. Dr. Leventhal stated that Dr. Sobol's comments confirm her opinion that this protocol should not have been approved. To approve any protocol, there must be an assurance that every patient who participates in an experiment, including novel therapy, will provide valuable data about that therapy. Investigators cannot discover efficacy in the course of treating a single patient. The fact that the investigators have no plan to study other patients confirms that no valuable information will be gained. Dr. Royston stated that they would be evaluating the patient's immune response to this treatment.

Dr. Leventhal said that if the study had been presented as the first of a series of patients, she would have recommended approval of this single patient protocol. In that way, valuable scientific information could have been derived from the experiment. Dr. Royston responded that he would like to obtain approval for the treatment of additional patients; however, the FDA required that this request to be submitted as a single patient IND. Dr. Leventhal said that the RAC will certainly review single patient protocols; however, this protocol is inadequate.

Dr. Royston stated that he is concerned by the fact that if he had submitted this study as a single patient protocol through the normal RAC review process, that it probably would have been deferred by the committee. Dr. Sobol said that he shares Dr. Royston's perspective. Dr. Walters stated that the RAC would welcome the review single patient and multiple patient protocols submitted by Drs. Sobol and Royston in the future, and that the committee would attempt to be fair in its review of such protocols based on their merits.

XVIII. ADJOURNMENT

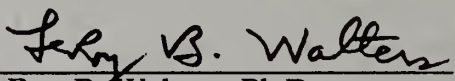
Dr. Walters adjourned the meeting at 4:02 p.m. on March 2, 1993.



Nelson A. Wivel, M.D.
Executive Secretary

I hereby acknowledge that, to the best of my knowledge, the foregoing Minutes and Attachment are accurate and complete.

Date: 6/7/93



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HUMAN GENE TRANSFER PROTOCOL

9303-037 Culver, Kenneth W., Iowa Methodist Medical Center, Des Moines, Iowa, and Van Gilder, John C.; University of Iowa, Iowa City, Iowa; *Gene Therapy for the Treatment of Malignant Brain Tumors with In Vivo Tumor Transduction with the Herpes Simplex Thymidine Kinase Gene/Ganciclovir System.*

Date of RAC Approval: 3/1/1993
Date of NIH Approval: 4/16/1993

Project Title: Gene Therapy for the Treatment of Malignant Brain Tumors with In Vivo Tumor Transduction with the Herpes Thymidine Kinase Gene/Ganciclovir System.

Principal Investigators: Kenneth W. Culver, M.D. and John Van Gilder, M.D.

Scientific Abstract

Murine retroviral vectors can infect a wide variety of proliferating mammalian cell types (e.g. lymphocytes). Non-proliferating tissues (e.g. neurons) are not transduced by murine retroviral vectors. These findings suggest that this type of vector may be useful for the selective introduction of genes into growing tumors in the brain, since the tumor is essentially the only tissue that will integrate and express the vector genes.

We have investigated the possibility of direct in vivo gene transfer into growing brain tumors in animals. Rats with a malignant brain tumor were given an intratumoral stereotaxic injection of murine retroviral vector-producer cells (VPC) that were producing vectors containing the Herpes Simplex-thymidine kinase (HS-tk) gene or a control VPC line containing the β -galactosidase gene. The animals were rested 5 days to allow time for the HS-tk retroviral vectors that were produced *in situ* to transduce the neighboring proliferating gliosarcoma. The animals were then treated with the anti-herpes drug ganciclovir (GCV). Tumors injected with the HS-tk VPC regressed completely with GCV therapy while the tumors injected with β -galactosidase VPC developed large tumors. Staining for β -galactosidase (+) cells in control animal brain revealed transduction of 10-50% of the tumor cells without evidence of transduction of the surrounding normal brain tissue. No significant toxicity was observed in toxicity studies in mice, rats and non-human primates.

Based upon these findings, we have proposed a human clinical trial to determine if the direct implantation of the G1TkSvNa VPC line into growing human brain tumors will induce regression with GCV therapy. The patient population primarily consists of individuals with recurrent malignant tumors who are currently considered ineligible for the approved NIH trial due to the size of the tumor. The RAC has previously approved an initial trial at NIH involving the stereotaxic injection of brain tumors. In this protocol, patients will undergo surgical debulking of the tumor followed by repeated treatments of HS-tk VPC into the tumor bed in an attempt to induce complete regression of the tumor with GCV therapy. These are patients who have failed standard therapy for their tumor and are expected to survive for several weeks to a few months.

Project Title: Gene Therapy for the Treatment of Malignant Brain Tumors with In Vivo Tumor Transduction with the Herpes Thymidine Kinase Gene/Ganciclovir System.

Principal Investigators: Kenneth W. Culver, M.D. and John Van Gilder, M.D.

Non-technical Abstract

We have investigated the possibility of transferring a "sensitivity" gene into a growing brain tumor. The purpose is to make the tumor sensitive to a type of chemotherapy that is relatively non-toxic to the rest of the body. The gene we have selected is the Herpes Simplex-thymidine kinase (HS-tk) gene. Herpes Simplex is a virus that can be killed by a drug called ganciclovir (GCV). By transferring the HS-tk gene into the tumor, using a disabled mouse virus called a "vector", we then essentially convert the tumor to be like a herpes virus and now the tumor can be killed with GCV.

Experiments in rats have shown that the direct injection of mouse cells producing a HS-tk vector into a growing brain tumor can result in complete destruction of the tumor with GCV therapy. We found no evidence of spread of the virus to the normal brain tissue or to other parts of the body. Based upon these findings, an initial trial is underway at the National Institutes of Health (NIH). To date, one person has been treated without evidence of toxicity. We now proposed a second human clinical trial for patients who are currently ineligible for treatment in the NIH trial due to the large size of their tumor. In this study, patients will undergo surgery to remove as much of the tumor as possible followed by repeated treatments of HS-tk vector-producer cells (VPC) into growing their human brain tumors in an attempt to induce regression of the tumor with GCV therapy. The patient population consists of individuals who have failed standard therapy and have recurrent primary or metastatic brain tumors with an expected survival of weeks to a few months.

CLINICAL RESEARCH PROJECT

Date: 3 January, 1993

To: Recombinant DNA Advisory Committee

Recommended by: Keith McRoberts, Ph.D., Director Research and Innovations, IMMC

Project Title: **Gene Therapy for the Treatment of Malignant Brain Tumors with
In vivo Tumor Transduction with the Herpes Simplex
Thymidine Kinase Gene/Ganciclovir System.**

Identifying Words: gene transfer, retrovirus, brain metastasis, glioblastoma

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Company Sponsor:

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Collaborator:

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Subject of Study	Number	Sex	Range
Patients	40	M or F	≥ 18 years

Project Uses Ionizing Radiation: ~~X~~
Yes No

Project involves use of Durable Power of Attorney ~~X~~
Yes No

Multi-institutional Project: ~~X~~ (Approvals secured)
Yes No

1. SPECIFIC AIMS

To apply gene therapy to intracranial tumors we propose a method for in vivo gene transfer of the Herpes Simplex thymidine kinase (HS-tk) gene using G1TkSvNa.29 vector-producing cells. Insertion of the HS-tk gene confers a sensitivity to the anti-herpes drug ganciclovir (GCV). We have demonstrated that the direct injection of HS-tk vector-producer cells (VPC) into growing tumors in animals can result in their complete destruction of the tumor with GCV therapy. This selective destruction of growing tumors in situ is thought to result from the transfer of the HS-tk gene into the surrounding tumor cells where the production of toxic GCV metabolites within the tumors result from the interaction of HS-tk and GCV. This procedure can result in the cure of some experimental animals with limited toxicity due to selective gene transfer into tumors. Therefore we propose to apply this technique for the treatment of human primary and metastatic brain tumors.

This clinical trial will focus on maximizing the relative number of VPC to the tumor mass, along with repeated administration of VPC in an attempt to maximize the anti-tumor effect. Adult patients (≥ 18 years) with surgically accessible brain tumors will be evaluated for the extent and location(s) of their disease before being entered onto study. Patients will undergo craniotomy and subtotal resection of the tumor. The tumor will then be directly injected with HS-tk VPC, leaving an Ommaya reservoir in place. Seven days later additional VPC will be injected through the Ommaya reservoir into the tumor bed. One week later GCV will be administered at 5mg/kg/dose IV BID for 14 days. Two weeks following the completion of the GCV administration additional VPC will be instilled. This second cycle of VPC will be given through the Ommaya reservoir 35 days after operation and 7 days later, the patients will undergo another cycle of intravenous therapy with GCV at 5mg/kg/dose BID for 14 days. The patient may receive further cycles of VPC and GCV depending upon the initial tumor, response, toxicities, clinical status and the radiographic evaluation of their tumors. The patient will then be followed every 2-4 weeks after the completion of the final course of GCV.

This protocol differs from the RAC and FDA approved NIH protocol primarily in the surgical technique. Therefore, this protocol may represent a minor modification of the NIH protocol. However, we felt it most appropriate to present a new protocol for RAC consideration since it will be conducted at two new institutions. The NIH trial focuses on the use of stereotaxic surgery with one treatment of multiple injections into a small tumor. Patients with larger tumors will likely require multiple treatments of VPC for there to be a possibility of complete tumor destruction. Larger tumors will likely have a higher fraction of more slowly dividing cells which will require repeated exposure to VPC for more complete transduction in vivo. Our proposal utilizing surgical debulking and the repeated administration of VPC without the need for repeated craniotomy offers an approach for patients who are currently ineligible for the NIH trial due to large tumor size.

Overall Aims and Objectives:

The study was designed to provide information on these aspects of this therapy:

1. Is there efficacy associated with the repeated administration of vector producing cells followed by GCV treatment in brain tumors?
2. Is there toxicity associated with the repeated administration of vector producing cells followed by GCV treatment in brain tumors?
3. Does the patient develop any evidence of systemic immunity as a result of the repeated injection of murine cells?

2. Background and Significance

I. Brain tumors and metastasis:

Brain tumors are a major cause of morbidity and mortality in the population. New primary brain tumors develop in approximately 15,000 adult Americans each year. They comprise the third leading cause of death from cancer in persons 15 to 34 years of age (1). Recent evidence indicates that the prevalence of primary brain tumors is increasing, especially in the elderly (2). The astroglial brain tumors, including the highly malignant glioblastoma multiforme (GBM), are the most common primary brain tumors. Despite aggressive therapy which includes surgical removal of the tumor and post-operative high dose radiation, the prognosis of patients with GBM is very grim with a median survival of 9 to 10 months (3). Although controversial, neither the quality nor time of survival is significantly improved when chemotherapy is added to surgery and radiation (4). When glioblastoma multiforme recurs, there is 100% mortality within weeks to a few months. In one study, a mean survival of 9 months was found in patients with recurrent GBM who underwent a second operation but, a reasonable quality of life in those patients was limited to 10 weeks following the recurrent GBM (5). Cerebral metastases are a frequent complication of systemic cancer occurring in 25 to 35 percent of the 1.1 million new cases of cancer per year in the U.S. (6). In 50% of patients, the metastatic disease is localized to the central nervous system (7). The cancers that most frequently metastasize to the brain are from melanoma, lung, breast, colorectal and renal cell cancers (8). A subset of patients may even be cured of their primary cancers only to succumb to the isolated metastatic disease in the brain. Surgery, combined with radiation therapy, is the treatment of choice for a single focus brain metastasis that is surgically accessible. Median survival using bimodality therapy (surgery and radiotherapy) reaches 40 weeks. In most patients with metastatic disease to the brain, multiplicity of the lesions, or their inaccessibility, prohibits surgical intervention and limits therapy to radiation alone with a median survival of about 15 weeks (9).

Recently published work suggests the feasibility of *in vivo* HS-tk gene transfer for the treatment of malignant brain tumors (10). The central nervous system has several advantages of safety and efficacy for retroviral mediated *in vivo* gene transfer. First, retroviral vectors only integrate and therefore express vector genes in proliferating cells. In the brain, the tumor is the most mitotically active cell, with only macrophage-derived cells, blood cells and endothelial cells at minimal risk. Therefore, the possibility of specific transduction of

the tumor is enhanced. Second, the brain is a partially immunologic privileged site, which should allow a somewhat longer survival of the xenogeneic murine cells in the brain and a greater transduction frequency of the growing tumor. This feature is further increased since human gliomas are known to further depress local immunity. This is thought to be secondary to a down regulation of IL-2 secretion and diminished expression of high affinity IL-2 receptors on T-lymphocytes (11). The murine cells should survive sufficiently long in the brain to allow for the transduction of greater numbers of tumor cells. However, this period of survival will be limited since all cells that integrate and express HS-tk will be destroyed by the GCV or eventually destroyed by the immune system.

II. Retroviral-mediated Gene Transfer (12,13).

In contrast to chemical and physical cellular transfection methods, murine retroviral vectors have proven to be extremely efficient for gene transfer into mammalian cells, with efficiencies as high as 90% in cultured murine fibroblast cell lines. Murine retroviral vectors differ from the adenoviruses and herpes simplex viruses in that the retroviruses will only integrate and subsequently express their genes in proliferating tissues (e.g. tumors). This feature of the retroviral vectors may be particularly advantageous in the brain, where the tumor is the predominant mitotic cell type, maximizing specific transduction of tumor with minimal, or absent, transduction of normal brain. The Moloney murine leukemia virus-based (MoMLV) vectors have been designed to minimize the possibility of recombination resulting in regeneration of a replication-competent virus (14,15).

In 1989, the first gene transfer experiment in humans was conducted at the NIH. This study involved the treatment of 10 patients with autologous T-cells (TIL) that have been transduced with a retroviral-vector. None of these patients have demonstrated any untoward effects secondary to receiving the genetically-altered cells (16).

Human gene therapy for adenosine deaminase deficiency has been performed on 2 children since the protocol opened in September, 1990. Twenty-three intravenous infusions of genetically altered autologous T-cells have been administered to date (17,18). Neither child has demonstrated any evidence of adverse effect due to the genetically altered cells.

3. Preliminary Studies

I. Pre-clinical Data

A. In Vivo Transduction with NeoR and β -galactosidase Vectors:

1. In Vivo Transduction of the NeoR Vector (LNL6) into the MCA 205 Murine Fibrosarcoma (See Appendix A: Reprint 1)(10,27). Initial studies were performed in mice to determine if tumor cells could be transduced in vivo. The retroviral vector LNL6, which has a titer of 1.0×10^6 cfu/ml, was utilized for these experiments. LNL6, which contains a NeoR gene promoted by the 5'-LTR, is free of replication-competent retrovirus. NeoR protects mammalian cells from the toxic effects of the neomycin analog G418. LNL6 VPC or control cells not producing vector but expressing the NeoR gene (LNL6 transduced 3T3 cells) and MCA 205 tumor were mixed in vitro and injected subcutaneously in syngeneic

C57BL/6 mice. Mice were inoculated with 1×10^6 MCA 205 tumor cells mixed with either 1×10^6 control 3T3-LNL6 expressing cells or with 1×10^6 LNL6 vector producing PA317 cells. The cells were mixed just prior to subcutaneous injection. Tumors measured twice weekly.

To minimize the possibility of contamination of the recovered tumor cells with 3T3 and PA317 cells, we waited 4 weeks before excising the growing tumors. The excised tumors were minced and digested into a single cell suspension. Tumor cells from each group were cultured for two weeks. The cultured cells were then placed in a clonogenic assay as shown in Table 1. (Appendix A: Reprint 1). The number of colonies that grew in medium without G418 were similar in each group with a cloning efficiency of 15-20%. There were no colonies with resistance to G418 in the tumor plus control 3T3-LNL6 expressing cell group. While the tumor plus VPC group had a mean of $62\% \pm 15$ (range 55-73) G418-resistant colonies. An assay for NPT activity, the enzyme produced by NeoR, was positive for all G418-selected tumors in the VPC group. In control mice, PA317/LNL6 producer cells alone produces a transient tumor, that is rejected in 7-10 days. Since the evaluation of tumors in the experimental group was performed at 4 weeks, it is very unlikely that these G418 resistant cells are the producer cells. The lack of G418-resistance in controls and the lack of LNL6 vector production by the recovered G418-selected cells suggests that the PA317/LNL6 producer line is not responsible for the G418-resistance.

2. In Vivo Transduction of the E. coli LacZ (β -galactosidase gene) Vector(G1BgSvNa) into the 9L Rat Brain Tumor. To evaluate the in vivo transduction dynamics within brain tumors, we have used the G1BgSvNa.29 vector. G1BgSvNa.29 (produced by Genetic Therapy Inc., GTI) has a titer of 1.5×10^6 cfu/ml. This vector contains NeoR and the E.coli derived gene LacZ which encodes for the production of the enzyme β -galactosidase (B-GAL) (19). The B-GAL expression can be detected using an X-GAL histochemical stain. Staining the brain with X-GAL turns B-GAL expressing cells blue. This results when an indolyl is liberated from X-GAL by the action of the B-GAL enzyme. Subsequent oxidization and self-coupling forms an indigo blue derivative. The vector containing cells can thus be discriminated from unmodified cells and then be enumerated with light microscopy. Rats were inoculated with 4×10^4 9L gliosarcoma cells into the right cerebral hemisphere by stereotaxic guidance. Seven days later, 3×10^6 PA317/G1BgSvNa VPC were injected into the tumor bearing and non-tumor bearing hemispheres using the same stereotaxic coordinates. Five, 9, and 14 days after injection of the VPC, the rats were sacrificed by an intracardiac injection of formaldehyde to fix the brain. The brains were stained with X-GAL. Control rats received G1BgSvNa.29 transduced, G418 selected 3T3 non-producer cells. The VPC and the control G1BgSvNa.29 transduced 3T3 cells were 100% positive by X-GAL staining prior to injection. In this experiment, we have shown that the injection of VPC led to transduction of 10-50% of the tumor cells in situ. Figure 2 (Appendix A: Reprint 2) presents the microscopic sections taken from the rats on day 7 and 14. The injected VPC disappeared from the injection site after day 14. There was a clear cut delineation between the transduced tumor cells and normal brain tissue. Except for the possibility of a rare transduced endothelial cell

in the vicinity of the tumor, no evidence of non-tumor brain transduction was evident in either cerebral hemisphere as shown in Figure 3 (Appendix A: Reprint 2). The most mitotically active endothelial cells in the area of the tumor are likely to be those responding to neovascularization signal within the tumor. Destruction of transduced endothelial cells with GCV therapy within the tumor may enhance the anti-tumor activity. The non-producer control cells did not demonstrate transduction of either tumor cells or normal brain and they were not seen in the tumor or brain after 14 days. This experiment demonstrates that local injection of a VPC line will transduce tumor cells in vivo, that the transduced tumor infiltrates surrounding brain with the non-transduced tumor and that in vivo retroviral-mediated gene transfer does not significantly effect adjacent normal non-proliferating brain tissue and that the producer cells disappear after 14 days.

B. In Vitro GCV Sensitivity of Mammalian Cells +/- Transduction with a HS-tk Vector

The G1TkSvNa.29 vector was transduced into the cell lines listed in Table 1, Appendix E in vitro using filtered supernatant from confluent producer cells. The transduced cell lines were then selected in G418 for 7 days at 1.0 mg/ml. Only cells expressing a functional NeoR gene are able to survive these G418 culture conditions producing an essentially 100% selected population of vector transduced cells. We then evaluated the sensitivity of the transduced, G418-selected cells compared to the non-transduced parent cell lines. In each case, the HS-tk-transduced, G418 selected cell lines were markedly more sensitive to low concentrations of GCV. Concentrations of 0.5-5 $\mu\text{g/ml}$ were inhibitory to the HS-tk-transduced cells in this assay. In a clonogenic assay, HS-tk positive cells were completely inhibited at 0.5 $\mu\text{g/ml}$. These findings confirm that HS-tk gene-containing retroviral vectors can effectively transduce murine and rat tumor cells and stably express both the NeoR and HS-tk genes resulting in 100% kill of the transduced cells in vitro when exposed to GCV. All in vivo studies were done using G418-selected VPC and control non-producer cell lines that had similar patterns of GCV sensitivity conferred by a transferred HS-tk gene.

C. Toxicity studies:

1. Assessment of HS-tk-producer toxicity in the peritoneal cavity and in the lung.

We have injected PA317/HS-tk VPC and control PA317/B-GAL VPC IP into mice (Appendix C). Twelve mice received 5×10^6 HS-tk VPC and 12 received 5×10^6 B-GAL VPC. The mice were observed for 7 days during which no evidence of toxicity was observed in either group. GCV was then administered at a dose of 150mg/Kg BID for six days. During and after GCV administration, no toxic side effects were observed. Six mice in each group were sacrificed at the end of GCV treatment. No gross or microscopic pathology was seen in various abdominal organs. The remaining 6 mice per group have been followed for > 12 months with no evidence of long-term toxicity. Next, 12, mice each received, 5×10^5 B-GAL or HS-tk VPC IV via the lateral tail vein to evaluate possible toxicity to the lungs where the cells are trapped. No evidence of toxicity was observed before, during and following GCV administration. Review of microscopic slides of the lungs revealed no areas of necrosis or other pathology in comparison to the control group.

2. Assessment of Transduction of Non Tumor Proliferative Tissues. Using the B-GAL gene as a marker, rat 9L brain tumors were injected with the PA317/G1BgSvNa.29 VPC. Organs were harvested and stained with X-GAL in order to estimate the frequency of vector expressing cells. Organs evaluated included the heart, lungs, liver, spleen, kidney, and small and large bowel. Organs were examined at 5, 9, and 14 days following the intra-tumoral injection of the vector. Organs from rats in which control non-producer B-GAL cells were injected into their tumors served as controls.

no X-GAL positive cells were seen in the heart and kidney. Occasional X-GAL positive cells were seen in the spleen, liver, and in the lungs, compatible with the distribution of normal macrophages which can produce positive results in this assay. No difference was observed between the frequency of X-GAL positive cells in these organs between the rats injected with the VPC and those injected with the non-producer cells. In normal bowel, there are a large number of X-GAL positive cells within the villi of both the small and large bowel. Again, no significant difference was observed between the non-producer and the VPC treated rats. These findings are consistent with the histology and suggest no significant spread of the vector takes place beyond the normal brain from the site of inoculation.

3. Toxicity studies of HS-tk VPC with and without GCV in normal brain.

Ten Rats were inoculated with 3×10^6 PA317/HS-tk VPC into the deep white matter of the cerebral hemisphere (Appendix D). The contralateral hemisphere served as control with 3×10^6 B-GAL-transduced 3T3 cells (3T3-B-GAL). Cells were injected in a volume of $50 \mu\text{L}$. Rats were treated with GCV at a dose of 15mg/Kg BID for 7 days and sacrificed 3 days later for histological evaluation of the brain. The deep injection site was evident in both hemispheres with local changes secondary to the injection of cells. No difference was seen between the HS-tk VPC injection site and the B-GAL-transduced 3T3 cell injection site. Surrounding brain tissue did not show evidence of inflammation or destructive changes in either group. Dexamethasone was administered to the rats as an oral dose of 0.5 mg/Kg/day, starting on the third day post injection of the VPC. It appeared that dexamethasone pretreatment diminishes the non-specific symptoms related to the surgery and implantation of cells in the brain.

4. Toxicity studies in non-human primates. Five monkeys (*Macaca mulata*, 6 - 8 Kg) were used for the primate experiments. The study was designed to address 2 major issues: 1. What is the fate (survival time, proliferation potential) of the murine VPC within the brain? 2. Is there significant toxicity from the intracerebral injection of HS-tk vector-producer cells, alone or with subsequent GCV therapy?

As is in standard neurosurgical practice, the monkeys were treated with high-dose steroids (dexamethasone, 2 mg/Kg IM/day) starting 7 days before surgery and continued for 3 weeks at which point the dose was gradually tapered during one week and discontinued. Antibiotic therapy (Cefotaxime, 30 mg/Kg, IM BID) was administered starting on the day of surgery and continued for 10 days. Using general anesthesia (IV pentothal to effect, Isoflurane 0.75% - 2.0% by inhalation) under aseptic conditions, monkeys received stereotaxic intracerebral injection of 10^7 HS-tk VPC mixed with 10^6 B-GAL VPC (total

volume of 250 μ L) into the deep white matter of the right frontal lobe. One monkey received bilateral injections of an HS-tk/B-GAL vector-producer cell mixture (10:1), as described, into the right frontal lobe and 10^7 BAG-transduced 3T3 cells into the left frontal lobe. Injections were done with a 250 μ L Hamilton syringe over 15 minutes.

A venous port was installed in two monkeys for subsequent GCV therapy via the right internal jugular vein. The proximal catheter was placed in the right atrium and the distal access port in the interscapular subcutaneous region on the back of the monkeys. GCV was administered to the two monkeys with the venous access port as a slow IV. infusion of 10 mg/Kg in 50 mL normal saline over 30 minutes for 14 days. MRI scans, including T1 and T2 weighted images and gadolinium-enhanced T1 weighted studies were obtained before GCV treatment (day 5 after cell injections), 7 days after initiation of GCV therapy (14 days after cell injection), and 90 days after cell injection in non GCV-treated monkeys. Physical and neurological examinations were performed twice a day on each monkey. Repeat blood samples were obtained from all animals before and during the experiment for routine chemistry and CBC. Cerebrospinal-fluid (CSF) samples were taken by cisternal puncture from all monkeys on the tenth post-operative day and were assayed for routine chemistry and bacteriological analysis. Two monkeys who received intracerebral injections of the HS-tk producer cells without GCV have now been followed nearly one year without evidence of adverse effects. These results are summarized in table 2, Appendix E.

- a. Fate of the producer cells: To address this question, histologic examination was carried out on the brains of 3 monkeys. No proliferation of the VPC within the brain was observed in either the GCV-treated (2) or non-treated (1) monkeys. This was determined on the basis of histological and radiological (MRI) examination of the brains. In one GCV treated animal examined 15 days after cell injection, some residual murine cells (using the B-GAL-producer cells as a marker) were found among the injected HS-tk VPC. No B-GAL positive murine cells were found in the 2 animals examined 3 weeks after cell injection, regardless if GCV was or was not administered. It is concluded that the VPC survive in the brain up to 15 days and cannot be seen thereafter. This time frame is similar to what we have observed in the rat brain.
- b. Toxicity Evaluation: Toxicity was evaluated by clinical, radiological, and laboratory (chemical, bacteriological, and histological) evaluation of the monkeys. Since the xenogeneic cells may cause an inflammatory response by leaking into the subarachnoid space (reactive meningitis), cerebrospinal fluid samples were collected from all monkeys 14 days after injection of the cells to assess that risk. All CSF samples were obtained by cisternal puncture and showed normal protein and glucose levels without pleocytosis. Bacterial cultures were negative. Table 3, Appendix E, summarizes the CSF characteristics of the samples.
- c. Clinical evaluation: Neurologic examination of all monkeys, before or after cell injection and during GCV therapy was unchanged from baseline. No motor or behavioral changes were observed at any phase of the experiment. Two monkeys treated with HS-tk-producer cell injections without GCV therapy were followed for evidence of long-term toxicity. For

nearly 12 months following cell injection, no ill-effects or any changes from baseline neurological status have been observed.

- d. Radiological evaluation - MRI Studies: Both GCV-treated and non-treated monkeys MRI scans showed the localized injection site as an isointense lesion (7 days after cell injection) or hypointense lesion (7 days after start of GCV) measuring a few mm in diameter with enhancement of the surrounding rim following IV. injection of gadolinium. No edema or mass effect were noted in any of the monkeys. This appearance is compatible with a local breakdown of the blood-brain barrier, as is expected from the cell injection alone.
- e. Histologic studies: Mild reactive gliosis was seen in the vicinity of the cell injection site without evidence of edema or pathological changes in neighboring brain tissue. This was true for both the GCV-treated and non-treated animals. Specimens stained for myelin showed localized demyelination limited to the injection site which did not increase in size when GCV was given. A few endothelial cells adjacent to the injection site showed β -galactosidase activity as evidence of transduction with the B-GAL vector.
- f. Complications: The only complications observed were 2 septic complications related to the profound immunosuppression induced in the monkeys by high-dose steroids. One monkey (number 1), which had undergone implantation of the venous access port into the right heart, developed acute bacterial endocarditis with resultant *Staphylococcus aureus* septicemia and septic shock that led to its death 3 days after cessation of the prophylactic antibiotic therapy. This occurred 7 days after starting GCV therapy. An MRI study that was obtained 24 hours before death as well as macroscopic appearance of the brain and histology showed no evidence of CNS-related toxicity. Another monkey, which received bilateral injections (HS-tk-producer on one side and B-GAL-transduced 3T3 cells on the other side) and no GCV therapy, died on the 21st post-operative day (one day before planned sacrifice). Necropsy revealed bilateral interstitial pneumonia due to cytomegalovirus as well as systemic mycosis (*Candida albicans* cultured from all harvested organs) as cause of death. Again, no clinical or histologic perturbations of the CNS were observed in that monkey.

D. In Vivo Transduction with HS-tk Vectors in Mice and Rats:

1. Anti-tumor Effect of In Situ Injection with an HS-tk Vector-producer Cell Line on the In Vivo Growth of the MCA 205 Tumor in Mice

In order to determine if in vivo gene transfer technique can promote an increased anti-tumor effect, we used the PA317/HS-tk producer cell line. PA317/HS-tk contains a NeoR gene promoted by the 5'-LTR and an SV40 promoted HS-tk gene. Figure 1 (Appendix A: Reprint 1) depicts the effect of GCV on tumor growth in vivo in mice that were injected with 1×10^6 MCA 205 tumor cells plus 2×10^6 3T3-NeoR cells, NeoR VPC, 3T3/HS-tk or PA317 HS-tk VPC. The mice were ear tagged, cages coded and the tumors were measured twice weekly with a calipers in 3 dimensions. Tumor size is expressed as a volume (length x width x height). GCV treatment was initiated 4 days after injection of the cells and continued twice daily for 12 doses of 150mg/kg/dose.

All tumors grew well without GCV treatment. The growth of those tumors injected with 3T3-NeoR or NeoR VPC were not affected by GCV treatment. Non-producer 3T3/HS-tk

cells grew somewhat slower than the 3T3-NeoR cells during GCV treatment as shown in Figure 1 (Appendix A: Reprint 1). However, the tumors with PA317/HS-tk producer cells completely regressed with GCV treatment. These findings suggest that the transfer of the HS-tk gene by in vivo gene transfer can eradicate tumors in vivo. Injection of transducer cells alone is insufficient for tumor eradication.

2. Anti-tumor Effect of HS-tk Vector-producer Cell Line Implantation into Growing 9L Gliosarcoma in Rats

9L is a rat gliosarcoma cell line derived from the Fisher 344 strain. This brain tumor model has been well characterized (20). Injection of 4×10^4 9L tumor cells into the cerebral white matter of a rat results in 100% lethality by 4 weeks. Therefore, we have used this brain tumor model to evaluate in vivo HS-tk transduction and the subsequent anti-tumor response to GCV treatment. Fisher 344 male rats weighing 250-350 grams were anesthetized and placed in a stereotaxic apparatus. On day 0, we implanted 4×10^4 9L tumor cells in a volume of $50 \mu\text{L}$ into the right cerebral hemisphere. 5 days later, the same stereotaxic coordinates were used to introduce either saline or 3×10^6 PA317/HS-tk producer cells in $50 \mu\text{L}$ directly into the growing tumor. 5 days later, the rats began treatment with GCV at 150 mg/kg/dose twice daily. On the 5th day (10 days since inoculation of the tumor), the rats brains were examined for the extent of tumor growth. The rats treated with the HS-tk VPC and GCV are the only animals that experienced complete macroscopic elimination of the tumor (14 of 14 rats)(Appendix A: Reprint 1, Figure 2). Microscopic analysis revealed either no evidence of tumor (11 of 14 rats) or some residual, mostly necrotic, tumor in the tumor bed (3 of 14 rats). There was no evidence of vasculitis or destruction of normal tissues due to spread of the vector. This experiment further supports the data obtained with the G1NaSvBg.29 vector, that this in vivo transduction method appears to be without significant side effects and has substantial efficacy (10).

E. Bystander Effect. One of the unique features of the tumor rejection in the HS-tk system in mice is the observation that not all the tumor's cells must contain the inserted gene in order to be killed by GCV. In mice given a subcutaneous tumor in which 100% of the cells carry the HS-tk gene, complete tumor regressions were seen in 13 of 15 animals following GCV treatment. Interestingly, when tumors established from cell mixtures containing 50% HS-tk gene-modified cells mixed with 50% wild-type unmodified tumor cells were treated with GCV, almost all tumors (14/15) regressed. Even in situations where the mixed tumor contained 90% unmodified, wild-type tumor cells mixed with only 10% HS-tk modified tumor cells, complete regression of the cancer was observed with GCV treatment in 9 of 15 animals (Table 2, Appendix A: Reprint 1).

The mechanism of this "bystander tumor kill" is not yet completely understood. This may involve the production of toxic triphosphates produced by the interaction of thymidine kinase and GCV within the tumor leading to inhibition of DNA synthesis and death of replicating cells. This does not seem to involve generalized non-specific cellular toxicity since the overlying skin and other tissues surrounding these HS-tk treated tumors are not

injured while the tumors expressing the genes and the admixed wild-type tumor cells are completely destroyed.

The 9L rat gliosarcoma and the human glioblastoma cell lines U251 and A172 were also sensitive to this "bystander" effect in an in vitro mixing experiment. G1TkSvNa.29 transduced and non-transduced tumor cells were mixed at different ratios in 96 well microtiter dishes. GCV was added to the wells and 24 or 48 hours later, the cultures were pulsed with tritiated thymidine. These bar graphs depict a greater decrease in proliferation than would be expected at a GCV level of 5.1 $\mu\text{g/mL}$ in the medium (the numbers over the bars represent the percent of transduced tumor in the culture) (Appendix B: Figure 1). The 5.1 $\mu\text{g/mL}$ concentration is easily within the therapeutic range established in humans with a dose of 5 mg/kg/dose. Since it is unlikely that 100% of the tumor cells in the brains of our patients will be successfully gene-modified, this "bystander" effect is very important for the successful elimination of the tumor using this approach (See Appendix B: Figure 1). Analysis of human melanoma, colorectal, breast, lung and renal cell carcinoma cell lines reveal that all demonstrate the "bystander" effect in vitro.

Pre-Clinical Summary: The pre-clinical findings noted above suggest that the direct inoculation of a brain tumor with HS-tk retroviral vector-producing cells can mediate complete tumor rejection. This in vivo gene transfer methodology selectively alters the sensitivity of a tumor cell to chemotherapy. Treatment with GCV does not result in widespread damage to the host immune system like many forms of chemotherapy. Our animal studies have demonstrated no significant toxicity to normal brain or any of the proliferating non-CNS tissues, suggesting that the implantation of VPC in a brain tumor is not associated with non-specific systemic toxicity.

II. Clinical Data

One patient has been treated with the stereotaxic injection of HS-tk VPC and GCV infusion. No evidence of toxicity due to cell implantation or GCV administration has been observed as of Jan 4, 1993.

4. Research Design and Methods

I. Selection of Patients

A. Inclusion Criteria

1. Adults with malignant brain tumors (primary and metastatic) who failed standard therapy for their disease will be eligible to enter the study.
2. Patients must have surgical accessible lesions as estimated from the pre-treatment radiological evaluation. These decisions will be made by the PIs in accordance with the standards of care of neurosurgical practice.
3. Male or non-pregnant female patients $\geq$ 18 years of age. Women of child bearing potential must practice strict birth control to prevent pregnancy for the duration of the study. Men will be advised to use barrier protection for the duration of the study.
4. Possess the ability to give informed consent and express a willingness to meet all the expected requirements of the protocol for the duration of the study. (Appendix L)

5. Adequate baseline organ function as assessed by the following laboratory values before surgery:

- a. Adequate renal function with serum creatinine of ≤ 1.5 mg/dL or creatinine clearance ≥ 45 mL/min/m².
- b. Platelet count $\geq 100,000$ platelets/mm³
- c. Absolute neutrophil count ≥ 1000 /mm³
- d. Hemoglobin ≥ 8.5 mg/dL
- e. Normal Partial Thromboplastin Time (PTT), and ProThrombin Time (PT).
- f. Bilirubin ≤ 2.5 mg/dL, SGOT and SGPT $< 4X$ normal.
- g. Patients with a performance status of $\leq$ ECOG 3 will be excluded from the study. (see appendix H).

Patients admitted for study under this protocol will be registered with the principal investigator at IMMC and the Univ. of Iowa.

B. Exclusion Criteria:

Patients with the following conditions will be excluded from the study:

1. Acute infection. Active infection is defined as any acute viral, bacterial or fungal infection which requires specific therapy.
2. Neurological deterioration. Patients with an increased ICP who require prompt reduction of ICP and have a surgically inaccessible lesion will be excluded.
3. HIV positive patients.
4. Pregnant patients.
5. Severe systemic diseases such as severe ischemic heart or lung disease considered to be associated with an unacceptable anesthetic/operative risk.
6. Multiple tumors.

II. Clinical Evaluation

A. Preliminary Evaluation and Screening

The following evaluation must be completed within 30 days of surgery:

1. Before a patient can be entered into this study, the investigators must receive information from the referring physician(s) detailing the clinical history, general laboratory results, specific neurologic and radiologic evaluations, their diagnoses, and all previous therapies (Appendix I: Patient Screening Form).
2. Data substantiating the histopathological diagnosis must be received for review prior to participation in the study. If no recent tissue diagnosis is available, the nature of the space occupying lesion (e.g. tumor vs. radiation necrosis) will be determined by a stereotaxic biopsy of the lesion prior to cell injection.
3. Pretreatment general physical examination and a comprehensive neurologic evaluation (Screening).
4. HIV test (must be negative) (Screening).
5. β -HCG serum pregnancy test must be negative within 14 days of surgery (Screening).

6. Renal and hepatic blood chemistries (uric acid, calcium, phosphorus, magnesium, SGOT, SGPT, Alkaline Phosphatase, LDH, total bilirubin, BUN, creatinine, albumin, total protein, amylase, electrolytes, glucose)(Screening).
7. Urinalysis (Screening)
8. PT, PTT, fibrinogen (Screening)
9. CBC with differential and platelets (Screening).
10. Radiological Evaluation. Each patient will undergo an MRI and/or CT study of the brain with contrast enhancement if appropriate. Preliminary decisions regarding the eligibility of the patient for treatment, and the injections and/or procedures needed for each patient will be decided based on these studies (Screening).
11. CXR, EKG (Screening).
12. Immunologic evaluation.
 - a. In vitro lymphocyte proliferative responses to mitogen and antigens such as OKT3, PHA, Diphtheria, Tetanus, Candida albicans, alloantigen and murine antigens (PA317 cell).
 - b. Cellular phenotype of peripheral blood by FACS analysis
 - c. Isohemagglutinins and quantitative immunoglobulins (A,G,M and E)
 - d. Serum for antibody to PA317 cells.
 - e. Soluble IL-2R
 - f. DTH skin test panel (CMI Multitest or equivalent)
 - g. Non-serologic HIV test
13. Freeze baseline sera samples and peripheral blood mononuclear cells.

B. Evaluations during the treatment period

1. Before GCV treatment. Patients will be evaluated daily throughout the study. The neurological exam will be recorded daily. CT with contrast or MRI scan of the brain will be obtained within 48 hours post-operatively depending on the clinical status of the patients and scan availability, and again within 48 hours of the infusion of producer cells through the Ommaya reservoir. Freeze serum sample and peripheral blood mononuclear cells.
2. During GCV treatment. Patients will be admitted to the hospital for GCV therapy, for a minimum of 48 hours until more information is gained about its use in this patient population. A neurological exam will be recorded daily. An MRI or CT scan with contrast will be performed at or near the end of the 14 day GCV therapy. Creatinine clearance tests, liver function tests, CBC, coagulation studies and blood chemistry will be monitored. Freeze serum sample and peripheral blood mononuclear cells.

C. Evaluation following the treatment period.

1. Follow up. A complete neurological and physical examination will be recorded prior to discharge. Patients will be seen as outpatients at 2 week intervals for the first 2 months and on a monthly basis for one year. Neurological status will be recorded at each follow up visit as well as a CT or MRI scan with contrast. The number of visits after the first year will depend on the status of the tumor.
 - a. CBC differential, platelet count

- b. Renal and hepatic blood chemistries (uric acid, calcium, phosphorus, magnesium, SGOT, SGPT, Alkaline Phosphatase, LDH, total bilirubin, BUN, creatinine, albumin, total protein, electrolytes, glucose)
 - c. Physical examination with a detailed neurologic exam.
 - d. β -HCG serum pregnancy test will be repeated monthly for women with child bearing potential while on therapy.
 - e. Radiological evaluation: Patients will have a CT scan or MRI of the brain (if needed) with contrast enhancement if appropriate.
 - f. Freeze serum sample and peripheral blood mononuclear cells.
2. Yearly follow-up laboratory evaluation. Patients will be requested to be followed once a year for the rest of their life for retroviral gene transfer safety monitoring.
- a. CBC with Differential count
 - b. Repeat Immunologic evaluation.
 - c. Serum for antibody to PA317 cells.
 - d. PCR on peripheral blood mononuclear cell DNA for vector sequences
 - e. Western blot analysis of serum for antibody to retroviral antigens
 - f. If at any time a new malignancy develops, we shall attempt to obtain involved tissue for analysis for the vector DNA.
3. It is understood that the performance of an individual study or test as specified in this protocol is subject to factors such as patient compliance, scheduling difficulties, equipment malfunction, or the clinical judgment of the principal investigator or patient care physician, and that a test may not be done in an individual instance with no violation of the protocol. However, any systematic modification of the original protocol in this regard, whether related to patient safety or not, will be submitted to the IRB for approval.
4. Autopsy: An attempt will be made to perform a complete autopsy on any patient who dies during the study. Whenever possible, tissues from the brain, tumor(s) and/or bone marrow, will be evaluated for the presence of the G1TkSvNa.29 vector by PCR.
- D. Criteria for Response**
- 1) Non responders: No observable decrease in the size or progression of the tumor by CT scan and/or MRI scan.
 - 2) Minimal responders: 25-49% decrease in tumor volume by CT scan and/or MRI compared to the size on post-op day 7 of 4 weeks duration.
 - 2) Partial responders: $>50\%$ decrease in tumor volume by CT scan and/or MRI compared to the size on post-op day 7 of 4 weeks duration.
 - 3) Complete responder: No remaining tumor on CT scan and/or MRI at 3 months of 4 weeks duration.
- E. Off-Treatment Criteria**
- Criteria for going off of therapy: The major criterion for suspending enrollment of patients into the protocol would be evidence of significant toxicity that is directly related to the application of our experimental therapy. Such toxicity may include the following:
- a. Irreversible diffuse cerebral dysfunction (motor, sensory, cognitive).

- b. Irreversible regional cerebral injury that is not related and cannot be explained by the physical aspects of the surgical procedures alone.
- c. Permanent Grade 3 toxicity or recurrent grade 4 toxicity as specified in Appendix J of the protocol. It should be noted that the assessment of neurological toxicity (Neurosensory, neuromotor, neurocortical, neurocerebellar, neuro-mood, neuro-headache, etc. as appears in Appendix J - Toxicity Criteria) relates to the relative changes from the pre-treatment neurological status. If such toxicity is observed in 2 of the first 3 patients, the study will be suspended and reevaluated. If no such toxicity is observed in the first 3 patients, but later is seen, the study will be abandoned.
- d. Other anticipated toxic phenomena that are not related directly to the in situ transduction of the brain tumor (such as toxicity secondary to GCV therapy), or neurological complications which can be explained by the presence of the mass lesion and are compatible with the natural history of malignant brain tumors, will not be considered a sufficient indication for the termination of the study. Any additional occurrence of toxicity will be discussed with the Chairman of the IRB.
- e. Upon request of the participating patient or the person with the power of attorney the patient may withdraw from the study.

III. Data Collection and Monitoring

- A. Study Monitoring.** Site visits will be done by GTI to monitor the data in the research records and to ensure that all regulatory requirements surrounding the study are met. The investigator will allow study monitors and the FDA to inspect study documents (e.g. consent forms, certificate of analysis, case report forms, and pertinent hospital or clinical charts).
- B. Reporting of Adverse Reactions.** (ADRs) to the IND Drug will be reported promptly to the FDA, IRB and IBC. ADR reports are required even if only a suspicion of a drug effect. Previously unknown Grade 2 or 3 reactions will be reported in writing within 10 working days. Grade 4 (life-threatening) reactions and patient deaths while on treatment will be reported by phone within 24 hours. A written report will follow within 10 working days.
- C. Protocol Amendment Procedures.** Any revisions to the original protocol will be discussed by all investigators, the FDA and others as required. If the consensus is to revise the current protocol, a formal list of changes will accompany the amended protocol and these will be submitted to the FDA, the site's IRB and other committees.
- D. Publications resulting from Trial.** Any manuscript, abstract, or presentation will be available to all the study investigators involved in this protocol and the sponsoring company for review prior to submission.

IV. Nature of Procedures or Therapeutic agents (See schema in Appendix F)

- A. Surgical Procedures.** A craniotomy will be performed and complete tumor removal will be attempted (day 1). The lining of the cavity will then be infiltrated at multiple sites with the HS-tk VPC (day 1) to a maximum volume of 5 mL. An Ommaya reservoir, which provides an access port to the brain, will be placed into the tumor bed.
- B. Use of the Ommaya Reservoir.** Seven days after the initial surgery, the Ommaya reservoir will have sealed into the patients brain and surrounding tissues. On day 7 the patient will

undergo a brain scan to estimate the volume of the tumor fossa. On day 8 the skin overlying the Ommaya will be cleansed with betadine. A needle will be inserted through the overlying skin into the reservoir with the patient awake. VPC will be slowly injected into the reservoir until the tumor fossa has been filled based on brain scan estimates of the tumor fossa volume. GCV will be administered starting on the 15th post-operative day for 14 days (days 15-28). Repeat administration of producer cells will be done on day 42, followed by another cycle of GCV administration 7 days later (days 49-63).

- C. **Volume and number of injected cells:** Factors such as tumor size, location, and the preoperative neurological condition of the patient will determine the injectable volume. The final cell concentration will be adjusted to $1-2 \times 10^8$ cells/mL.
- D. **Peri-operative medications:**
 1. Antibiotics: All patients will receive a single dose of Ceftriaxone or the equivalent just prior to the initial surgical procedure and prior to the injection of the Ommaya reservoir.
 2. Steroids: All patients will receive dexamethasone at 32 mg/day starting 7 days prior to each VPC injection and treatment will be continued until GCV is discontinued. Dexamethasone will then be tapered. As experience is gained, the need for high dexamethasone dosing may be modified.
 3. Mannitol: Patients may receive mannitol during the surgical procedure at 1g/kg and the dose repeated TID for 24 hours following the procedure, as clinically indicated.
 4. Anticonvulsants: Anticonvulsive therapy will be administered according to the usual neurosurgical guidelines.
 5. Analgesics: Pain medications will include Acetaminophen 650-1000 mg Q 4 hours.
- E. **The G1TkSvNa.29 Retroviral Vector** (Appendix G). G1TkSvNa.29 is a retroviral vector derived from the Moloney murine leukemia virus (MoMLV). This vector contains a Herpes simplex type I thymidine kinase (HS-tk) gene cDNA that is transcribed from the viral LTR and a bacterial neomycin resistance (NeoR) gene transcribed from an internal SV40 (simian virus 40) early promoter (LTR--HS-tk--SV--NeoR--LTR) in the G1 vector backbone (Genetic Therapy Inc., Gaithersburg, Md). This G1-based vector has been modified for increased safety by alteration of the gag start codon to a stop codon, and by elimination of viral sequences needed in trans for the formation of the virus particle. This has been shown to minimize the potential for the development of replication-competent virus production from producer cells which contain the vector. The HS-tk gene is a negative selectable marker or "suicide" gene. When a HS-tk transduced cell is exposed to GCV, the GCV acts as a substrate for phosphorylation by HS-tk resulting in a monophosphate (MP) form of the drug. Cellular phosphorylases convert this GCV-MP to GCV-triphosphate (GCV-TP) that inhibits DNA polymerase and is incorporated into DNA resulting in an inability of the cell to proliferate. The end result is cell death for the HS-tk transduced cells (21-25). The NeoR gene is a positive selectable marker. The bacterial NeoR gene encodes for NPT II (neomycin phosphotransferase II), an enzyme that will protect G1TkSvNa.29 expressing cells from the toxic effects of G418 (a neomycin analog). NeoR has been used in many human clinical trials to date without adverse effect. NPT-II inactivates the antibiotic

Amikacin but does not inactivate other aminoglycoside antibiotics (such as gentamicin and tobramycin) (26). Due to the availability of a large number of antibiotics for use in the treatment of gram negative infections, the NeoR gene should not affect the management of gram negative infections.

F. Preparation of the G1TkSvNa.29 Vector-Producer cell line. The generation of retroviral vectors from transinfected PA317 cells has been extensively tested in vitro and in human gene transfer/therapy experiments. The G1TkSvNa transinfected PA317 cells were selected in G418 and cloned. Clone 53 has produced the highest NeoR and HS-tk titer (5×10^5 colony forming units/mL for both) and this clone has been used in clinical trials at the NIH. It has been shown to be free of detectable replication competent virus.

G. Growth of the G1TkSvNa.29 Vector-Producer cell line for Clinical Use

1. The producer cells are grown in complete medium which contains Dulbecco's modified MEM with 4.5 gm glucose/liter with L-glutamine (DMEM), 10% FCS, 2 mM L-glutamine.
2. Monolayer Culture (flasks and roller bottles): The monolayer cultures are grown in T-75 flasks at 37°C with 5% CO₂. When the cells are >90% confluent, the cells are trypsinized and split 10:1 in fresh complete medium. The cultures are scaled to yield sufficient cells for clinical trials. A cell bank, working cell bank, and a product cell bank has been prepared by GTI, and have been shown to be free of detectable adventitious agents (See Appendix K).

H. Testing and Harvest of the Producer cell line for Clinical Use

1. An aliquot of supernatant will be sent for a STAT gram stain and culture.
2. The cells will be trypsinized, harvested into 50 mL conical tubes (Falcon), washed three times in normal saline and counted by trypan dye exclusion.
3. The final cell concentration will be adjusted to $1-2 \times 10^8$ cells/mL.

Tests done on the product lot used for clinical administration are listed in Appendix K

V. Potential Hazards Implanting of G1TkSvNa.29 VPC into Human Brain Tumors

A. Potential surgical complications:

Infection: Patients with malignant brain tumors have a significant predisposition to a variety of superimposed infections secondary to a state of immune suppression as previously described. The probability of post-operative wound infection is also increased due to previous radiation damage to the scalp following radiotherapy. Antibiotic therapy will be given as a prophylaxis prior to the surgical interventions and as medically indicated.

Increased ICP: Patients with an intracranial space occupying lesion often present with symptoms attributable to increased intracranial pressure (ICP). All the patients in this study will be treated prophylactically with high dose dexamethasone before the surgical procedures. Additional measures (Mannitol) may be given in the peri-operative period.

Steroid complications: The long term effects of steroids are well documented. However, most of the patients who will enroll in our study will require steroids for control of increased ICP regardless of the proposed treatment. Inducing a reduction in tumor size may actually allow the reduction of the steroid dose in some patients.

Chemical meningitis: Despite the lack of a meningeal reaction in our model laboratory animals, meningitis or meningitis-like symptoms may develop secondary to spillage of the

VPC into the subarachnoid space. Such symptoms are expected to be self-limiting and ameliorated with symptomatic care (analgesics). However, such a reaction may be severe and could produce severe permanent neurological deficits or death. When indicated, CSF sampling may be required to rule out infection.

Severe edema: Edema secondary to necrosis and destruction of the transduced tumor cells may occur. The magnitude of such edema, if it occurs at all, is unknown. As a precaution, all patients will be treated with high-dose steroids. If edema around the tumor is not responsive to therapy, permanent, significant neurological deficits or death may result.

Surgical Procedures: The surgical procedures carry a risk for loss of neurological function, non-neurological complications and death. The risk depends on the preoperative condition of the patient, size and location of the tumor, and associated diseases (e.g. Ischemic heart disease, renal failure, COPD etc.). The individual risk will be determined prior to the decision on the line of therapy and will be discussed with the patient prior to surgery.

- B. **Use of the Ommaya Reservoir.** The Ommaya reservoir is a commonly used device in neurosurgery. The potential complications resulting from its use are infections and bleeding. Every effort will be used to maintain sterility and prevent bleeding.
- C. **The development of replication-competent retrovirus.** As discussed (Section IV.E.).
- D. **Survival of Vector Producer Cells in the Host.** The PA317 cells can survive only 7-14 days in a subcutaneous site in syngeneic (H-2b) mice and only 7-14 days in xenogeneic rat brain. Likewise in immunosuppressed monkeys, the producer cells could not be identified > 14 days after injection. Taken together, these findings suggest that any PA137 cells that are not killed by GCV will not have the ability to survive long term in the human brain.
- E. **Dissemination of G1TkSvNa.29** Our MoMLV amphotropic retroviral vectors are directly inactivated by human complement without antibody. Therefore, escape of free vector into the cerebral spinal fluid or the vascular space should result in immediate inactivation. The gene transfer in this direct injection system is most likely due to the intimate contact of the tumor and VPC. Any vector particles that are released in the area of injection will be quickly bound by the thousands of amphotropic vector receptors on each tumor cell and other host cells. Vectors binding to non-dividing cells in the brain will be lost. Even if all of the vector particles produced were able to escape direct transfer into adjacent tumor cells or binding to non-dividing cells and cross the blood-brain barrier, the number of vector particles relative to the number of receptors in any organ would still be very small suggesting a minimal risk of injury to proliferating cells in any non-CNS organ. Any direct transfer into neurons by cell-to-cell contact will not result in HS-tk gene integration and therefore, should not pose a risk for their destruction with GCV treatment.
- F. **Transduction of surrounding brain tissue.** There is no evidence in our animal model that transduction of surrounding normal brain tissue is likely to occur (Section 3.I.A.2.). However, if the HS-tk gene is introduced into a large number of normal dividing cells within the CNS (such as endothelial cells and astroglial cells), vasculitis like symptoms (headaches, convulsions, bleeding) may develop. Such changes however will be localized to the immediate vicinity of the tumor as had been shown in our experiments (3.I.C.2).

G. Insertional Mutagenesis. Retroviral vector DNA is inserted randomly into the genome of proliferating cells. The random nature of this integration has the potential of an untoward insertional event. If the insertion disrupts a gene essential for maintaining cell function, that particular cell will die. Since the gene transfer will occur most predominantly in tumor cells, the vector insertion site will result in the death of a few tumor cells without GCV, which should not pose a problem. The risk of oncogenic transformation with these replication-incompetent retroviral vectors cannot be accurately estimated since that has never been a documented occurrence in animals or man. While this is a real risk, this risk must be very low, especially in this protocol, where all vector containing cells will be killed by GCV within 2-3 weeks of injection of the producer cells. Based upon available data, the risk of death secondary to their tumor far exceeds any risk of insertional mutagenesis.

H. Ganciclovir sodium (GCV; Cytovene®). The GCV used in this trial will be obtained from Syntex corporation (Palo Alto, CA). GCV is an approved drug for cytomegalovirus (CMV) retinitis therapy in immunocompromised individuals. The drug is given by IV infusion over 1 hour (dose of 5 mg/kg) twice daily for 14-21 days. FDA approved guidelines (where applicable), administration procedures, drug interactions, and patient monitoring will be followed. A copy of the Cytovene® product monograph has been provided to the Recombinant DNA Activities office. There is minimal information regarding the use of GCV for the treatment of humans as a method to destroy HS-tk gene transduced human cells. Further information about toxicity is being generated in the initial NIH trial. To date, no toxicities have been noted in the first patient. GCV has been noted to cross the blood-brain barrier. The cerebral spinal fluid (CSF)/plasma ratio has been estimated in 3 patients at various time intervals with ratios ranging from 0.24 to 0.7 (0.31-0.68 $\mu\text{g/mL}$ in the CSF and 0.44-2.20 $\mu\text{g/mL}$ in the plasma). Peak plasma levels have been documented to reach 9 $\mu\text{g/mL}$. These CSF and plasma levels are expected to be within the range of GCV levels needed to kill the HS-tk transduced cells based on in vitro studies (0.5 $\mu\text{g/mL}$ will prevent growth of HS-tk-transduced tumor cells). If the patient has evidence of renal impairment, the dose will be adjusted as suggested in the GCV monograph.

Granulocytopenia (absolute neutrophil count (ANC) of ≤ 1000 cells/ mm^3) in 40% of patients and thrombocytopenia ($\leq 50,000$ platelets/ mm^3) in 20% are the most frequent side effects. This data was collected in CMV infected AIDS patients, who may have been more susceptible to marrow suppression, due to other opportunistic infections or drug therapy. The actual risk to our patients is unknown. Each patient will be closely monitored for the development of granulocytopenia and thrombocytopenia. The development of an ANC of < 500 cells/ mm^3 or a platelet count of $< 25,000$ platelets/ mm^3 will require a dose interruption or decrease until the ANC is > 750 cells/ mm^3 platelet count is $> 40,000$ platelets/ mm^3 . Other side effects occurring in approximately 2% of patients include anemia, fever, rash and abnormal liver function. Fever and chills will be treated with acetaminophen (650 mg every 4 hours) and rash with diphenhydramine (50mg every 6 hours). See the monograph for complete toxicity criteria. Our treatment protocol will use the known tolerated dose (10mg/kg/day) in non-infected patients for only 14 days.

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Medical Record : Consent to Participate in a Research Study
Iowa Methodist Medical Center

Study Number: _____ Principal Investigator: Kenneth Culver, M.D.

CONSENT TO PARTICIPATE IN A RESEARCH STUDY: GENE THERAPY FOR THE
TREATMENT OF MALIGNANT BRAIN TUMORS WITH IN VIVO TUMOR TRANSDUCTION
WITH THE HERPES SIMPLEX THYMIDINE KINASE GENE/GANCICLOVIR SYSTEM

FEDERAL REGULATIONS REQUIRE WRITTEN INFORMED CONSENT FROM PARTICIPANTS PRIOR TO PARTICIPATION IN A RESEARCH STUDY SO THAT THEY CAN KNOW THE NATURE AND RISKS OF PARTICIPATION AND DECIDE TO PARTICIPATE OR NOT TO PARTICIPATE IN A FREE AND INFORMED MANNER. YOU ARE ASKED TO READ THE FOLLOWING MATERIAL TO ENSURE THAT YOU ARE INFORMED OF THE NATURE OF THE RESEARCH STUDY AND OF HOW YOU WILL PARTICIPATE IN IT IF YOU CONSENT TO DO SO. SIGNING THIS FORM WILL INDICATE THAT YOU HAVE BEEN INFORMED AND THAT YOU GIVE YOUR CONSENT.

INTRODUCTION

There is no effective treatment for malignant brain tumors that recur following surgery, radiation therapy and/or chemotherapy. Therefore, we have developed a new experimental approach, for the treatment of recurrent brain tumors, that takes advantage of knowledge gained in the fields of genetics and gene therapy. A virus that causes mouth sores and other types of infections is called herpes simplex. It can be treated with a drug called Cytovene. The Herpes simplex virus is killed by Cytovene because the virus contains a specific gene called the Herpes-thymidine kinase gene (TK gene). The TK gene has been isolated in the laboratory and in experiments has been injected into brain tumors in rats. This causes the brain tumor cells containing the TK gene to be killed when the rats are treated with Cytovene. In some of the rats treated this way, the brain tumors disappeared. The purpose of this study is to gain information about whether or not this new approach is effective in treating human brain tumors. Although the findings in animals are encouraging, you will be one of the first humans to receive this investigational treatment for brain tumors. While we hope to gain information on the usefulness of this approach in humans, we cannot know whether your tumor will get smaller as a result of this treatment. It is possible that this treatment could make you worse.

What is gene therapy for brain tumors?

We will attempt to change the genetic material of your brain tumor cells by inserting the TK gene into the tumor cells. The way we will transport the TK gene into the tumor cells is to use a "vehicle" to carry the TK gene into the cells. An example is that if you want to move a passenger you need to put him in a car. The vehicle (or car) in this case a different virus - a retrovirus that is

found in mice - that has been inactivated so that it can not cause disease. We call this vehicle a "vector". We insert the TK gene (the passenger) into the vector and the vector carries the gene into your brain tumor cells. The vector/gene combination is produced by "vector-producer" cells (little factories that produce the cars containing the passengers). When the vector/gene combination gets into your tumor cells it inserts itself into the cells' genetic material (chromosomes) and tells the tumor cells to begin making thymidine kinase. We will also attach to the vector vehicle a bacterial gene that we think is harmless; this gene serves to mark the location of the TK gene in your body. When we give you Cytovene, the tumor cells containing the TK gene produce harmful substances within themselves that cause the tumor cells to die. We do not expect normal brain cells to be injured because the vector/gene combination is attracted only to rapidly growing cells. Normal cells are not rapidly growing but tumor cells are and are therefore targets for the vector/gene combination.

Pretreatment Evaluation:

You may be admitted to the hospital for approximately 1 week for the pretreatment evaluation and the following procedures. You will have a complete neurological and physical examination, blood, and urine tests to determine your overall physical health. The amount of blood drawn from you will be no more than three ounces in one day and will not exceed one pint over your stay at the hospital. You may experience some discomfort or temporary pain at the site of the needle entry for the routine blood studies. There is remote risk of fainting and local infection.

You will also have x-rays of your chest and head and any other areas of the body as indicated by your medical examination and history. An electrocardiogram will be performed. Additional tests may be required depending upon your state of health and prior therapies. The blood tests will also include a test for the HIV (AIDS) virus. Women of childbearing age will also have a pregnancy test. Patients with HIV and pregnant women will not be eligible for this protocol.

An MRI scan and/or a CT scan of your brain will be done. Both scans are designed to examine the changes in the nervous system that occur with the recurrence of the brain tumor. MRI uses a strong magnetic field and radio waves to show structural and chemical changes in the brain tissues. A CT scan uses x-rays. The type of scan chosen will depend upon the status of your tumor and the response to this therapy. During the scan, a needle will be placed into a vein in your arm and an image-enhancing dye may be administered through that vein before obtaining a second set of scans. Each scan takes about 60 to 90 minutes. Scans will be repeated periodically after injection of the vector producing cells to monitor the effects of this therapy on tumor growth.

TREATMENT PHASE

The size and location of your tumor are such that partial surgical removal is possible. Therefore you will undergo three procedures in the following order. 1. You will undergo brain surgery to remove as much of the tumor as possible with injection of vector-producing cells into areas of the remaining tumor. An Ommaya reservoir will be placed under the scalp with a tube connecting the reservoir to the tumor site. The reservoir allows for the repeated injection of drugs and cells without repeated surgery on the brain. 2. One week after surgery, we will inject additional vector-producer cells through the Ommaya reservoir into the tumor cavity. 3. You will receive twice daily intravenous injections of Cytovene for 14 days. This portion of the study is expected to require a 3-4 week hospitalization.

Surgery:

You will be taken to the operating room and, following the administration of general anesthesia, a craniotomy (direct surgery on the brain) will be performed. A piece of skull will be removed, the coverings of the brain will be opened and as much as safely possible of the brain tumor will be removed. Since it is impossible to completely remove a recurrent brain tumor, the remaining tumor will be injected with the vector producing cells during the operation. Following surgery, you will be monitored in the intensive care unit (ICU). During the various stages of therapy, you will receive various drugs to prevent convulsions and swelling within the brain. These drugs are used routinely in neurosurgery and are given to almost all patients who undergo brain tumor surgery. Antibiotics will be given to you on each day of surgery.

Injection of vector-producing cells through the Ommaya reservoir:

Seven days after surgery additional vector-producing cells will be injected into the area of the remaining tumor. This will be performed in the clinic by the neurosurgery staff. There is no need for anesthesia.

Cytovene administration:

Fourteen days after surgery, Cytovene infusions will be started. Cytovene will be administered into a vein by a slow drip lasting about one hour. The drug will be given two times a day for 14 days. You may have an MRI or CT scans before, during and after Cytovene therapy.

Follow-up Care and Evaluation

After you will finish the course of Cytovene you will be discharged from the hospital. Two weeks after discharge, you will be required to return as an outpatient for follow-up studies. You will undergo neurological and physical examinations and laboratory tests, as were performed before your surgery. An MRI or CT scan of the brain will also be obtained. Following the initial visit, you will be seen as an outpatient at 2 week intervals for 2 months and monthly for 10 months. The number of visits after the first year will depend on the status of your tumor. We expect that we will receive one

sample of blood per year for the remainder of your life to monitor the safety of this gene transfer methodology.

RISK ASSOCIATED WITH EVALUATION AND TREATMENT

Risk of MRI and CT scans:

MRI and CT brain scans are painless procedures that are very safe for most patients. During the scan you will lay flat in a long metal cylinder, which people who fear closed spaces sometimes find a little frightening. Patients can be observed at all times by the operators and can be assisted if necessary. You will be moved out of the machine if requested. The MRI machine makes a thumping sound while operating.

A small proportion of people develops short-lived reactions during the dye administration including nausea, headache, hot flashes, and heart palpitations. These symptoms usually resolve spontaneously within minutes. A smaller group of patients are actually allergic to the dye and may develop a rash, hives, respiratory difficulty, and in extreme cases pulmonary and cardiac arrest and death. You will be closely monitored and if an allergic reaction develops, you will be promptly treated with epinephrine and breathing assistance if necessary. Both therapies are immediately available in the room where the brain scan will be performed.

Patients at risk for injury from MRI are those with pacemakers, aneurysm clips (metal clips on the wall of a large artery), or shrapnel fragments. Welders and metal workers are also at risk for injury because of possible metallic foreign bodies in the eye. If you are at risk for injury by MRI scanning, you will undergo CT scanning instead.

Risk of Surgery:

The surgical risk depend on the preoperative condition of the patient, the nature of the operation, and the location and size of the tumor. Known risk associated with brain surgery include: hemorrhage, deterioration of neurological functions (such as weakness in the arm and/or leg, loss of sensation over parts of your body, and partial or complete loss of functions related to communication such as speech and comprehension, and other functions related to intellectual capacity, memory, etc.), infection and death. The relative risk will be discussed with you in accordance with your condition, specific findings and planned surgical procedures.

Risk of the Ommaya reservoir:

The Ommaya reservoir is a commonly used device in neurosurgery. The Ommaya reservoir is inserted under the skin with an attached tube that extends into the area of tumor. To use the Ommaya reservoir, the overlying skin is cleansed and a needle is inserted through the skin into the chamber.

The potential complications relating to the use of the Ommaya reservoir are infection and bleeding. Every effort will be made to maintain sterility and prevent bleeding.

Risk of the Vector/TK Gene Transfer:

Even though the vector producing cells cannot grow and are considered harmless in humans, it is possible that events could occur within the cells that would permit the vector to grow and/or make the cells cancerous. Gene transfer using similar vectors has been used in adult and child patients. Since these experiments started in 1989, none of the more than 20 people who have received cells into which genes had been transferred by vectors has developed any problems related to the gene transfer method. We believe these vectors are safe and are not a threat to other people or to society.

This method of treatment has 2 major potential problems. First, the vector may be passed into surrounding normal tissue in addition to tumor tissue. We have not found any evidence of problems in mice and rats due to the spread of vector to surrounding normal brain tissue or to other sites in the body. We believe that some of the surrounding blood vessel cells probably do have the vector, but the number is too small to result in significant adverse side effects. It is possible that bleeding and neurologic symptoms (headache, convulsions, stroke) may develop with Cytovene.

Second, the vector producing cells might persist in your body and cause cancer or other diseases. We expect your immune system to reject (kill) the vector producing cells in 1-2 weeks. Thus, they should not be able to survive and grow in your body. In addition, we expect the Cytovene therapy will kill all cells with the vector, including the vector producing cells. Therefore, the vector producing cells should not survive and the insertion of the vector should not result in new cancerous cells, since we think all of the cells with the vector will be killed by Cytovene. Therefore, we feel that the risk of developing a new cancer is very small.

Risk of Cytovene Therapy:

Cytovene has been used extensively in humans to treat a number of infections including viral infections of the eye. We will be following the recommended dose for therapy that has been used to treat viral infections of the eye. The most commonly observed complication in people receiving Cytovene has been the development of decreased white blood cell and platelet counts. This could result in an increased risk of infection and bleeding. We will monitor these counts every other day (during Cytovene treatment) and the drug will be discontinued if the white blood count or platelet count drops significantly. Discontinuation of the drug results in normalization of the white blood cell and platelet numbers. Cytovene may cause permanent or temporary infertility and may be associated with birth defects. Therefore, women of childbearing age should use effective contraception during Cytovene therapy and men should use contraception during and for at least 90 days following

Cytovene therapy. Pregnant women are not eligible for this protocol. Cytovene can cause cancer in animals. There is no information available to estimate the risk of this in humans.

Additional Risk:

This procedure is relatively new and it is possible that despite our extensive efforts other unforeseen problems may occur including the possibility of unknown and possible disabling effects or death.

DURABLE POWER OF ATTORNEY FOR HEALTHCARE

You have an illness that at some point may impair your ability to think clearly and to make decisions. A Durable Power of Attorney for Healthcare is a process by which you appoint someone to make decisions about your medical care should you become, and only if you become, unable to make these decisions yourself. Such people might include a husband, wife, other family member or close friend. It is important that this person know your moral, religious and/or personal preferences in research and medical care. The Durable Power of Attorney for Healthcare form helps us give you medical care and assures that your preferences in research and medical care are respected, if you should be unable to give informed consent. A Durable Power of Attorney for Healthcare form can be obtained from your attorney or from the admissions office at Iowa Methodist Medical Center.

A TENTATIVE AGREEMENT TO AN AUTOPSY

In case of death during or after your participation in this research study, we ask you for a tentative agreement to an autopsy. This will provide us with an opportunity to learn more about the effect of our treatment of your tumor.

ALTERNATIVE TREATMENTS

You have been offered the opportunity to participate in this study after your tumor recurred despite appropriate standard therapies for your disease. There are no other standard treatments that were shown to have significant effects in patients with your disease. You may or may not have received chemotherapy for the treatment of your brain tumor. Chemotherapy is sometimes used for the treatment of primary brain tumors, but its effect is usually considered marginal. A variety of experimental studies for the treatment of brain tumors are conducted in medical centers around the world, but the benefit of their approaches is as yet unknown. In addition, there is always the possibility for you to decline any further treatment for your disease.

OTHER PERTINENT INFORMATION

1. When results of this study are reported at scientific and medical meetings or in journals, no patient will be specifically identified. Your medical records at Iowa Methodist Medical Center will be maintained in accordance with legal requirements and will be made available only as required by approved members of the Food and Drug administration, National Cancer Institute and Genetic Therapy Inc. unless your written approval has been obtained.
 2. This clinical procedure has attracted a great deal of attention from the media. We will make every effort to protect the confidentiality of you and your family. However, because of this media interest there is a greater risk than usual that information concerning your treatment will appear publicly without your consent.
 3. You are free to withdraw your consent to participate in this study and seek care from any physician at any time. This will involve no loss of benefits and will not penalize your care in any way.
 4. Iowa Methodist Medical Center will provide short-term medical care for any physical injury resulting from your participation in this research protocol. Long-term care or financial compensation for injuries is not otherwise offered as part of this study.
 5. There is no financial compensation for participation in this study. Payment for the costs of your participation in this study will be sought from entities involved in this research study or your third party payer(s).
 6. For answers to questions or should any problem arise regarding this research protocol, you should contact one of the following investigators.
Kenneth W. Culver, M.D. (Principal Investigator) (515) 241-6761
Thomas Carlstrom, M.D. (Neurosurgeon) (515) 241-5760
Charles J. Link, M.D. (Oncologist) (515) 241-6761
 7. We suggest you retain a copy of this document for future reference and personal records.
-

All of my questions have been answered to my satisfaction and I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby consent to take part in this study.

Signature of Adult Patient & Date Signed

Signature of Investigator & Date Signed

Signature of Witness & Date Signed

PATIENT INFORMATION SUMMARY

Project Title: Gene Therapy for the Treatment of Malignant Brain Tumors With In Vivo Tumor Transduction With the Herpes Simplex Thymidine Kinase Gene/Ganciclovir System

Investigator: John C. VanGilder, M.D.

Doctor _____ has explained to you that this is a study to evaluate the effectiveness of genetic therapy for treatment of your malignant brain tumor.

There is no effective treatment for malignant brain tumors which recur following surgery, radiation therapy, and/or chemotherapy. Therefore, a new experimental approach has been developed for the treatment of recurrent brain tumors that takes advantage of knowledge gained in the field of genetics and gene therapy.

A virus that causes mouth sores and other types of infection is called herpes simplex. It can be treated with a drug called Cytovene. Herpes Simplex is killed by Cytovene because the virus contains a specific gene called a herpes/thymidine kinase gene (TK gene). The TK gene has been isolated in the laboratory and in experiments has been injected into brain tumors in rats. This causes the brain tumor cells containing the TK gene to be killed when the rats are treated with Cytovene. In some of the rats treated this way, the brain tumors disappeared.

You are invited to participate in this study to gain information about whether or not this new approach is effective in treating human brain tumors. Although the findings in animals are encouraging, you will be one of the first humans to receive this investigational treatment for brain tumors. While we hope to gain information on the usefulness of this approach in humans, we cannot know whether your tumor will get smaller as a result of this treatment. It is possible that this treatment could make you worse.

What is gene therapy for brain tumors?

We will attempt to change the genetic material in your brain tumor cells by inserting the TK gene into the tumor cells. The way we will transport the TK gene into the tumor cells is to use a "vehicle" to carry the TK gene into the cells. An example is that if you want to move a passenger you need to put him in a car. The vehicle (or car) in this case is a different virus - a retrovirus that is found in mice - that has been inactivated so that it cannot cause disease. We call this vehicle a "vector". We insert the TK gene (the passenger) into the vector and the vector carries the gene into your brain tumor cells. The vector/gene combination is produced by "vector/producer" cells (little factories that produce the cars

containing the passengers). When the vector/gene combination gets into your tumor cells, it inserts itself into the cell's genetic material (chromosomes) and tells the tumor cells to begin making thymidine kinase (an enzyme). We will also attach to the vector vehicle a bacterial gene which we think is harmless: this gene serves to mark the location of the TK gene in your body. When we give you Cytovene, the tumor cells containing the TK gene make harmful substances within themselves that cause the tumor cells to die. We do not expect normal brain cells to be injured because the vector/gene combination is attracted only to rapidly growing cells. Normal cells are not rapidly growing, but tumor cells are and are therefore targets for the vector/gene combination.

Pretreatment Evaluation

If you decide to participate in this study, you will have a complete neurological and physical examination, blood and urine tests to determine your overall physical health. The blood tests will also include a test for HIV (aids) virus and women of childbearing age will have a pregnancy test. Patients with HIV and pregnant women will not be eligible for further study. In addition, appropriate x-rays and brain scans will be done before you begin the study.

Treatment Phase

This phase can be divided into three components.

Surgery: In the operating room following administration of general anesthesia, a craniotomy (direct surgery on the brain) will be performed. The brain tumor will be removed to the extent that is safely possible to do. Since it is always impossible to completely remove a recurrent malignant brain tumor, the remaining tumor and the brain immediately surrounding the tumor will be injected with the vector producing cells during the operation. You will receive various drugs that are used routinely in neurosurgery to prevent convulsions and brain swelling. These drugs are given to almost all patients who undergo brain tumor surgery. At the termination of surgery a small tube will be placed from under the scalp to the tumor bed (Ommaya reservoir).

Seven days after surgery additional vector producing cells will be injected into the area of the remaining tumor. This will be done through the Ommaya reservoir and is performed by the neurosurgical staff in the clinic. There is no need for anesthesia during this procedure.

Fourteen days after surgery Cytovene infusions will be started. Cytovene will be administered into a vein over a one hour period. The drug will be given two times a day for fourteen days. You will have a brain scan before, during and after Cytovene therapy.

Followup Care and Evaluation

After you have finished the course of Cytovene you will be discharged from the hospital. Two weeks after discharge you will be required to return to the neurosurgical clinic as an outpatient for followup studies. You will undergo neurological and physical examination as well as laboratory tests similar to those performed prior to your surgery. A brain scan will also be obtained. Following the initial visit, you will be seen as an outpatient at two week intervals for two months and monthly for ten months. The number of visits in the first year will depend upon the status of your tumor. We expect that we will receive one sample of blood per year for the remainder of your life to monitor the safety of this gene transfer methodology.

Study Risks

A small portion of people develop allergic reactions to the contrast agents given during brain scanning. These symptoms include nausea, headache, hot flashes, and heart palpitations. They usually resolve spontaneously within minutes. A smaller group of patients are actually "allergic" to the dye and may develop hives, rash, respiratory difficulty and in extremely cases pulmonary and cardiac arrest. You will be closely monitored and if an allergic reaction develops, you will be promptly treated with Epinephrine and breathing assistance if necessary. These therapies are immediately available in the room where the brain scan is performed.

The surgical risks depend upon the preoperative condition of the patient, the nature of the operation, and the location and size of the tumor. Known risks associated with brain surgery include hemorrhage, deterioration of neurological function (such as weakness of the arm and/or leg, loss of sensation over parts of your body, and partial or complete loss of functions related to communication such as speech and comprehension as well as function related to intellectual capacity and memory), infection and death. The relative risks will be discussed with you in accordance with your condition, specific findings, and planned surgical procedures.

The Ommaya reservoir is a commonly used device in neurosurgery. This reservoir is inserted under the skin with an attached tube that extends into the area of tumor. A needle is inserted through the skin into the chamber of the reservoir for injection of material into the tumor. The potential complications relating to the use of the Ommaya reservoir are infection and bleeding.

Risks of the Vector/TK gene transfer

Even though the vector producing cells cannot grow and are considered harmless in humans, it is possible that events could occur within the cells that would permit the vector to grow and/or make the cells cancerous. Gene transfer using similar vectors has been used in adult and child patients. Since these experiments began in 1989, none of the more than 20 people who have received cells into which genes have been transferred by vectors have developed any problems related to the gene transfer method. We believe these vectors are safe and are not a threat to other people or to society.

This method of treatment has two major potential problems. The vector may be passed into surrounding normal tissue in addition to tumor tissue. We have not found any evidence of problems in mice and rats due to spread of vector to surrounding normal brain tissue or to other sites in the body. We believe that some of the surrounding blood vessel cells probably do have the vector but the number is too small to result in significant adverse side effects. It is possible that bleeding and neurological symptoms (headache, convulsions, stroke) may develop with Cytovene.

The vector producing cells might persist in your body and cause cancer or other diseases. We expect your immune system to reject (kill) the vector producing cells in one to two weeks. Thus, they should not be able to survive and grow in your body. In addition, we expect the Cytovene therapy will kill all cells with the vector including the vector producing cells. Therefore, the vector producing cells should not survive and the insertion of the vector should not result in new cancerous cells since we think all the cells with the vector will be killed by Cytovene. Therefore, we feel the risk of developing a new cancer is very small.

Risks of Cytovene therapy:

Cytovene has been used extensively in humans to treat a number of infections including viral infections of the eye. We will be following the recommended dose for therapy that has been used to treat viral infections of the eye. The most commonly observed complication in people receiving Cytovene has been development of decreased white blood cell and platelet counts. This could result in an increased risk of infection and bleeding. These counts will be monitored every other day (during Cytovene treatment) and the drug will be discontinued if the white blood cell count or platelet count drops significantly. Discontinuation of the drug results in normalization of the white blood cell and platelet numbers. Cytovene may cause permanent or temporary infertility and may be associated with birth defects. Therefore, women of childbearing age should use effective contraception during Cytovene therapy and men should use contraception during and for at least 90 days following Cytovene therapy. Pregnant women are not eligible for this protocol. Cytovene can cause cancer in animals. There is no information available to estimate the risk of this in humans.

Finally, because this procedure is relatively new it is possible that despite our extensive efforts, other unforeseen problems may occur including the possibility of death.

You or your insurance will be responsible for any expense incurred during your treatment (clinic visit, labs, x-rays, or other medication).

We will keep any information we learn from this study confidential and disclose it only with your permission. By signing these forms you allow us to make records available to the Food and Drug Administration and Genetic Therapy, Inc. If the information we learn from this study is published or reported at scientific or medical meetings, you will not be identified by name.

Whether or not you take part in this study will not effect your future relations with your doctors or The University of Iowa Hospitals.

You understand that you may or may not directly benefit from treatment with this study.

Your participation in this study is voluntary. You may withdraw your consent and discontinue participation in this study at any time for whatever reason.

If you have further questions, you may contact John C. VanGilder, M.D. (319) 356-2772.

I have discussed the above points, including the information required by the Iowa Fair Information Practices Act, with the subject and his/her legally authorized representative, using a translator if necessary. It is my opinion that the subject understand the risks, benefits, and obligations involved in participation in this study.

(Signature of Investigator)

(date)

(Signature of Witness)

(date)

(Signature of Subject)

(date)

CERTIFICATION OF PATIENT CONSENT

Project Title: Gene Therapy for the Treatment of Malignant Brain Tumors with In Vivo Tumor Transduction with the Herpes Simplex Thymidine Kinase Gene/Ganciclovir System

Investigator: John C. VanGilder, M.D.

I, _____ hereby certify that I have been told by
(subject's name)

_____ of the Department of Neurosurgery about research
(investigator)

of malignant brain tumors and its purpose. I have been told about the procedures to be followed, which of them are experimental, and how much time is involved. I understand the possible risks and discomforts I may experience and the possible benefits to me and to others from the research. I have also been told the extent to which any records which may identify me will be kept confidential.

A written summary of what I have been told is attached. I have been given adequate opportunity to read it.

I understand that I have the right to ask questions at any time and that I should contact John C. VanGilder, M.D. at (319) 356-2772 for answers about the research and my rights.

I understand that in the event of physical injury resulting directly from the research procedures, no compensation will be available in the absence of negligence by a state employee. However, medical treatment is available at The University of Iowa Hospitals and Clinics, but I will be responsible for making arrangements for payment of the expenses of such treatment. Further information may be obtained from Dorothy M. Maher, Division of Sponsored Programs, Office of the Vice President for Research, (319) 335-2123.

I understand that my participation is voluntary, that I may refuse to participate or withdraw my consent and stop taking part at any time without penalty or loss of benefits to which I may be entitled.

I hereby freely consent to take part in this research project.

(signature of subject)

(date)

I, the undersigned, certify that I was present during the oral presentation of the written summary attached when it was given to the above subject.

(signature of auditor-witness)

(date)

CERTIFICATION OF SUBJECT CONSENT
BY LEGALLY AUTHORIZED REPRESENTATIVE

Project Title: Gene Therapy for the Treatment of Malignant Brain Tumors with In Vivo Tumor Transduction with the Herpes Simplex Thymidine Kinase Gene/Ganciclovir System.

Investigator: John C. VanGilder, M.D.

I, _____, the _____ of
(representative's name) (relationship or legal status)

_____ hereby certify that I have been told by

_____ of the Department of Neurosurgery about the research for malignant brain tumors and its purpose in which my _____ has been asked to take part. I have been told about the procedures to be followed, which of them are experimental, and how much time is involved. I understand the possible risks and discomforts, and the possible benefits relating to this research project. I have also been told about any appropriate alternative procedures that might be helpful to the subject. I have also been told the extent to which any records which may identify the subject will be kept confidential.

A written summary of what I have been told is attached. I have been given adequate opportunity to read it.

I understand that I have the right to ask questions at any time and that I should contact John C. VanGilder, M.D. at (319) 356-2772 for answers about the research and my rights.

I understand that in the event of physical injury resulting directly from the research procedures, no compensation will be available in the absence of negligence by a state employee. However, medical treatment is available at The University of Iowa Hospitals and Clinics, but I will be

responsible for making arrangements for payment of the expense of such treatment. Further information may be obtained from Dorothy M. Maher, Division of Sponsored Programs, Office of the Vice President for Research (319) 335-2123.

I understand that _____ participation is
(subject)
voluntary, and I may withdraw my consent for him/her to take part at any time without penalty or loss of benefits to which he/she may be entitled.

I hereby freely consent to _____
(subject's name)
taking part in the research project.

(legally authorized representative's signature)

(date)

I, the undersigned, certify that I was present during the oral presentation of the written summary attached when it was given to the above legally authorized representative of the subject matter.

(auditor-witness signature)

(date)

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME	POSITION TITLE
Kenneth K. Culver, M.D.	Clinical Director

EDUCATION (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
Drake University, Des Moines, Ia	B.A.	1973-77	Biology
University of Iowa, Iowa City, Ia	M.D.	1977-81	Medicine
University of California, San Francisco		1981-85	Pediatrics
University of California, San Francisco		1985-87	Fellow-Immunology

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the master's baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership in any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

National Institutes of Health, Bethesda, Md	1987-90 Clinical Associate
National Institutes of Health, Bethesda, Md	1990-92 Sr Clin. Investigator
Iowa State University, Ames Iowa	1992- Professor-Genetics
Iowa Methodist Medical Center, Des Moines, Ia	1993- Director-Res. Institute

Diplomat, American Board of Pediatrics (1986)
Diplomat, American Board of Allergy and Immunology (1989)

Honors and Awards

1977	Omicron Delta Kappa Honor Society
1977	Beta Beta Beta Biological Honor Society (President)
1981	Sandoz Award for outstanding clinical and research activities in Child Psychiatry
1986	Bank of America-Giannini Foundation Medical Research Fellowship
1990	Drake University Young Alumni Award
1991	BioEast '90 Prize for Outstanding Technical Innovation -- The production of high titer retroviral vector preparations using a hollow fiber cell culture system
1992	Harvard Health Letter - "First human gene therapy, the most important advance in medical research in 1991"

Membership in Professional / Scientific Societies

American Association of Immunologists
Clinical Immunology Society
American Association for the Advancement of Science
American Academy of Pediatrics

1. Culver, K.W., Ammann, A.J., Wara, D.W., Partridge, J.C., Wong, D.F., and Cowan, M.J.: Lymphocyte Abnormalities in Children Born to IV Drug Abusing Mothers. J Pediatr 111:230-5, 1987.
2. Cowan, M.J., Walker, C., Culver, K., Weintrub, P.S., Wara, D.W., and Levy, J.A.: Maternally Transmitted HIV Infection in Children. AIDS 2:437-41, 1988.
3. Culver, K.W., Smith, W.L., and Cowan, M.J.: Allogeneic Bone Marrow Transplantation in Newborn C57BL/6 Mice Treated Perinatally with Anti-Thy 1.2 Monoclonal Antibody. Transplant Proceedings. 21:2939, 1989.
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7. Culver, K.W., Able, C., Toper, R., Freeman, S., Berzofsky, J.A., and Blaese, R.M.: Lymphocytes as vehicles for gene therapy. Cellular Immunity and the Immunotherapy of Cancer. Eds: MT Lotze and O.J. Finn. Wiley-Liss, New York, pgs.129-137, 1990.
8. Blaese, R.M. and Culver, K.: Prospects for gene therapy of human disease. Allergol Immunopathol (Madr) 19:25-8, 1991.
9. Rosenberg, S.A., Abersold, P., Cornetta, K., Kasid, A., Morgan, R., Moen, R., Karson, E., Lotze, M.T., Yang, J.C., Topalian, S., Merino, M.J., Culver, K., Miller, A.D., Blaese, R.M., and Anderson, W.F.: Gene transfer into humans-Immunotherapy of patients with advanced melanoma, using tumor-infiltrating lymphocytes modified by retroviral gene transduction. N. Engl. J. Med. 323:570-578, 1990.
10. Blaese, R.M., Culver, K.W. and Anderson W.F.: The ADA human gene therapy clinical protocol. Hum. Gene Ther. 1:331-362, 1990.
11. Culver, K.W., Morgan, R.A., Osborne, W.R.A., Lee, R.T., Lenschow, D. Able, C., Cornetta, K., Miller, A.D., Anderson, W.F., and Blaese, R.M.: In vivo expression and survival of gene-modified Rhesus T lymphocytes. Hum. Gene Ther. 1:399-410, 1991.
12. Culver, K.W., Osborne, W.R.A., Miller, A.D., Fleisher, T.A., Berger, M., Anderson, W.F. and Blaese, R.M.: Correction of ADA Deficiency in human T-lymphocytes using retroviral-mediated gene transfer. Transpl. Proc. 23:170-171, 1991.
13. Culver, K., Cornetta, K., Morgan, R., Morecki, S., Abersold, P., Kasid, A., Lotze, M., Rosenberg, S.A., Anderson, W.F., and Blaese, R.M.: Lymphocytes as cellular vehicles for gene therapy in mouse and man. Proc. Nat. Acad. Sci. 88:3155-3159, 1991.
14. Culver, K.W., Anderson, W.F. and Blaese, R.M.: Lymphocyte gene therapy. Hum. Gene Ther. 2:107-109, 1991.
15. Blaese, R.M., and Culver, K.W. Progress toward the application of gene therapy. In Nance S.J., ed. Clinical and Basic Science Aspects of Immunohematology, Arlington, Va., American Association of Blood Banks, 1991: 1-11.
16. Culver, K.W., Blaese, R.M., and Anderson, W.F.: Gene Therapy. In the Encyclopedia Britannica Yearbook of Science and the Future, 1992.
17. Culver, K.W.: The Future of Gene Therapy. In Transgenic Animal Models in Biomedical Research Proceedings. Scarpelli, D.G., Migaki, G and Pletcheer, J.M. (eds). pg. 111-112, 1992.
18. Culver, K. W., Ram, Z., Walbridge, S., Ishii, H., Oldfield, E.H., and Blaese, R.M. In vivo gene transfer with retroviral vector producer cells for treatment of experimental brain tumors. Science. 256:1550-2, 1992.
19. Culver, K.W., R.M. Blaese, W.F. Anderson. Human gene therapy for adenosine deaminase deficiency. In press.
20. Ram, Z., Culver, K. W., Walbridge, S., Blaese, R.M., and Oldfield, E.H. In Situ Retroviral-mediated Gene Transfer for the Treatment of Brain Tumors in Rats. Cancer Research. In press.
21. Culver, K.W. The potential for genetic healing. JAMA 1992; 268:1768.

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME	POSITION TITLE		
VanGilder, John C.	Professor and Chairman		
EDUCATION (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)			8/14/35
INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
W. Virginia U., Morgantown, WV	B.A.	1957	
W. Virginia U. School of Medicine Morgantown, WV	B.S.	1959	Medicine
U. of Pittsburgh, Pitt., PA	M.D.	1961	Medicine

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

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HUMAN GENE TRANSFER PROTOCOL

9303-038

Heslop, Helen E.; Brenner, Malcolm K.; and Rooney, Cliona, St. Jude Children's Research Hospital, Memphis, Tennessee; *Administration of Neomycin Resistance Gene Marked EBV Specific Cytotoxic T Lymphocytes to Recipients of Mismatched-Related or Phenotypically Similar Unrelated Donor Marrow Grafts.*

Date of RAC Approval: 3/2/1993

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SUMMARY

Patients who receive bone marrow transplants from a mismatched or unrelated donor have a high risk of morbidity and mortality from viral infection during the period of immune deficiency which follows the procedure. One such problem that occurs in these patients is EBV lymphoproliferation due to outgrowth of EBV infected B cells that would normally be controlled by EBV specific cytotoxic T cells. This complication occurs in 5-30% of recipients of mismatched or unrelated transplants and is almost invariably fatal.

One possible approach to preventing this significant complication is to adoptively transfer EBV specific T cells from the BMT donor during the period when the recipient is at risk after the transplant. In this protocol we plan to evaluate this approach in a Phase I dose escalation study. Spontaneous lymphoblastoid cell lines which express the same range of EBV encoded proteins will be established at the time of confirmatory tissue typing and used as stimulator cells to derive EBV specific CTLs from donor blood obtained at the time of bone marrow harvest. Resulting cell lines will be characterized for EBV specificity and marked with the neomycin resistance gene. Marking these cells with the neomycin resistance gene is an important part of the study as it will allow us to learn how long these cells survive in the patient and whether expansion occurs *in vivo*. This information will be important in determining if the cells persist long enough to potentially confer protection and in determining appropriate doses and administration schedules.

NON-TECHNICAL SUMMARY

Bone marrow transplant using a mismatched or unrelated donor is the only curative treatment for patients with some types of leukemia. Patients who receive this type of transplant have a high risk of viral infections while their new marrow is growing back. One such problem that occurs in these patients is EBV lymphoproliferation where cells infected with the EBV virus grow in an uncontrolled manner. This complication is almost always fatal in such patients.

Outgrowth of EBV infected cells is normally prevented by T cells which specifically recognize EBV infected cells and kill them. We wish to determine if we can prevent this significant complication by generating EBV specific T cells from the BMT donor and infusing them during the period when the patient is at risk after the transplant.

Marking these cells with the neomycin resistance gene is an important part of the study as it will allow us to learn how long these cells survive in the patient and whether expansion occurs and therefore how long they may provide protection. This information will allow us to learn if this approach may be beneficial and what dosage regimens would be appropriate.

ADMINISTRATION OF NEOMYCIN RESISTANCE GENE MARKED EBV
SPECIFIC CYTOTOXIC T LYMPHOCYTES TO RECIPIENTS OF MISMATCHED-
RELATED OR PHENOTYPICALLY SIMILAR UNRELATED DONOR MARROW
GRAFTS

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1.0 CHECKLIST FOR PATIENT ELIGIBILITY PRIOR TO BMT

NO YES

- | | | |
|--------------------------|--------------------------|---|
| ☐ | ☐ | Age less than 21 years at time of diagnosis |
| ☐ | ☐ | Receiving matched unrelated donor or mismatched family member BMT for leukemia on or as per the MISMUD protocol |

CHECKLIST FOR ELIGIBILITY AT TIME OF ADMINISTERING EBV SPECIFIC CTLs

- | | | |
|--------------------------|--------------------------|--|
| ☐ | ☐ | Cytotoxic T cell line availability (Call Ext 2529 or 375) |
| ☐ | ☐ | No GVHD or Grade I GVHD |
| ☐ | ☐ | ANC $>500/\text{mm}^3$ |
| ☐ | ☐ | Bilirubin $<2 \times$ normal, SGOT ≤ 3 normal; prothrombin time normal |
| ☐ | ☐ | Creatinine <2 times normal for age or creatinine clearance >2 times normal for age |
| ☐ | ☐ | Normal electrolytes, calcium, phosphorus, nutritional status |
| ☐ | ☐ | No severe intercurrent infection |
| ☐ | ☐ | Life expectancy of at least 6 weeks and Performance status (ECOG) of 0-2. |

To check eligibility or register patient call Dr. Heslop at 531-2529 or Dr. Brenner at 522-0410.

- | | | |
|--------------------------|--------------------------|---|
| ☐ | ☐ | Informed consent explained to and signed by patient/guardian |
| ☐ | ☐ | Check table in Section 7.2 of this protocol to see necessary baseline and follow-up studies |

2.0 OBJECTIVES

- 2.1 To generate EBV specific cytotoxic T cell lines from BMT donors
- 2.2 To determine the safety of four weekly intravenous injections of BMT donor derived EBV specific cytotoxic T lymphocytes marked with the neomycin resistance gene in patients transplanted with marrow from a matched unrelated or mismatched family member for leukemia.
- 2.2 To determine the survival and immunologic efficacy of neomycin gene marked EBV specific cytotoxic T lymphocyte lines in bone marrow transplant recipients.

3.0 BACKGROUND AND RATIONALE

3.1 Viral Infections After Unrelated or Mismatched BMT

When allogeneic BMT is undertaken using a donor other than a matched sibling the increased alloreactivity between donor and recipient results in increased incidences of graft rejection and GVHD.(1-3). The risk of rejection can be reduced by increasing the conditioning to eliminate residual recipient alloreactive T cells and the risk of GVHD can be reduced by depleting the donor marrow of mature alloreactive T cells (4-7). Both these manoeuvres are effective and in the 24 St Jude patients receiving mismatched or unrelated donor BMT for leukemia in the last 2 years the Day 50 survival has been 100%. However as a consequence of GVHD and rejection prevention other problems occur in these patients.

Since August 1990, 24 transplants using mismatched family and closely matched unrelated donors, with in vitro T cell depletion, in patients with leukemia have been performed at St Jude. Thirteen of these patients have died and in nine (38%) the cause of death was a documented viral infection (4 with CMV pneumonitis, 3 with EBV lymphoproliferative syndrome, 1 with adenovirus hepatitis, 1 with bronchiolitis obliterans organizing pneumonia and positive culture for parainfluenza). In another two the cause of death was pneumonitis with no virus isolated. In addition many of these patients had other viral infections and the eleven surviving patients have also experienced considerable morbidity from viral infections. This experience is repeated at almost every other center performing these transplants (8). This increased risk of infection may be partly due to the immunosuppressive regimens these patients receive and to delayed immune recovery but may also relate to greater genetic disparity resulting in defective interactions between antigen presenting cells and immune system effector cells.

In most cases viral infection post BMT results from reactivation of latent virus. CMV has historically been the major cause of morbidity and mortality but the incidence of CMV pneumonitis has decreased since the advent of Ganciclovir and IVIG prophylaxis regimens (9,10). However the incidence of EBV lymphoproliferation post BMT is increasing as more transplants are performed from mismatched or unrelated donors and is now between 5 and 30% in this group.

3.2 EBV Biology in Normals

EBV is an ubiquitous human herpes virus which infects over 90% of normal individuals. In primary infection the main route of entry is the oropharyngeal epithelium. Replication of the virus in epithelial cells allows infection of B lymphocytes resulting in a polyclonal proliferation of transformed B cells. These B cells express a number of EBV gene products including the nuclear

antigens EBNA1, EBNA2, EBNA3A, EBNA3B and EBNA3C leader protein and the latent membrane proteins LMP1 and LMP2 (11). Although primary infection may be asymptomatic or associated with an infectious mononucleosis syndrome, in almost all cases the immune system controls the acute infection by recognizing the EBV generated antigens described above. The virus then persists for the life of the host in the latent state in B cells. EBV can be demonstrated in B lymphocytes in all positive individuals with an estimated frequency of 1 in 10^6 B cells. Epithelial cells provide an additional reservoir and in both these cell types EBV exists as a circular plasmid and expresses a limited set of genes. Outgrowth is prevented by immune system effector mechanisms including neutralizing antibodies to virus membrane proteins, HLA restricted EBV specific cytotoxic T cells and MHC unrestricted effectors. However when patients are immunosuppressed after organ transplants, reactivation may occur as evidence by increased viral replication in saliva and increased antibody titres (11,12).

3.3 EBV Disease in Transplant Recipients

As with other herpes viruses, reinfection with EBV is thought to result primarily from reactivation of latent virus. Studies have shown that there is increased viral replication in saliva and increased numbers of circulating EBV + B cells in patients who are immunosuppressed (11). In a BMT recipient EBV reactivation may arise from residual host B lymphocytes which have survived the conditioning regimen, from donor B lymphocytes or blood transfusions or from oropharyngeal shedding. Disease produced by reactivation of EBV has until recently been less common than disease produced by CMV, occurring with an incidence of around 1%. However the increasing number of patients receiving marrow from mismatched family or unrelated donors has resulted in an enormously increased incidence of EBV lymphoproliferative disease post BMT especially in regimens employing ATG post transplant (13). This increase is a consequence first of the increased immunosuppression these patients receive and also may reflect delayed immune reconstitution in these patients.

3.4 EBV Reactivation Produces Lethal Lymphoproliferation

The incidence of EBV lymphoproliferation following BMT from an MHC identical sibling donor is around 0.25% (14,15). The incidence following mismatched or unrelated BMT ranges from 5-30% in most series with the highest incidence seen in patients who receive the most immunosuppressive regimens.

The primary pathologic process is uncontrolled proliferation of mature B lymphocytes. Analysis of proliferating lymphocytes shows that these cells are usually of donor origin. The transformed B cells closely resemble morphologically and phenotypically the lymphoblastoid cell lines generated in

vitro when human B cells are infected with EBV in the absence of T cells. The cells have a immunoblastoid or plasmacytoid appearance and are usually CD19, CD20, CD21 and CD24 positive (12,16,17). Like Burkitt lymphoma cells, the lymphoblasts express EBV nuclear antigen 1 (EBNA1) but unlike Burkitt lymphoma cells they express all of the five other virus encoded latent cycle nuclear antigens and are positive for most latent membrane proteins. In addition they express a number of cell adhesion molecules. All these phenotypic features would make them intensely vulnerable to cytotoxic T cell killing in a normal individual and it is only the profound suppression of the immune system after transplantation which permits their outgrowth. The B cell proliferation is polyclonal in the early stages but eventually becomes oligoclonal or monoclonal.

Early in the illness fever and malaise are present. The proliferating lymphoblasts may then produce a number of disease patterns. They may diffusely infiltrate a number of organs including lung, liver, kidney, gut, bone marrow and central nervous system. Alternatively a classic lymphoma pattern is seen with lymphadenopathy, hepatosplenomegaly, and a biopsy appearance of immunoblastic lymphoma. Rarely a predominantly leukemic picture occurs. Initial reactivation of EBV associated with an infectious mononucleosis like illness may resolve. But once frank lymphoproliferation has occurred, the course is usually rapidly progressive.

As yet there is no effective treatment for EBV lymphoproliferative disorders. Withdrawal of immunosuppression which is effective in around 50% of the cases which follow transplantation of solid organs is rarely effective post BMT. Acyclovir may disrupt the replicative lytic cycle of the linear virus and interferon may prevent infection of fresh lymphocytes, but neither approach can significantly modify the growth of already transformed B cells which contain non replicating (circular virus). Recent reports suggest that infusions of monoclonal antibodies to CD21 and CD24 may eradicate infected B lymphoblasts in some patients with oligoclonal proliferations (18) but no effective treatment is available for monoclonal disease.

3.5 Importance of the Immune System in EBV Control

A number of lines of evidence suggest that EBV specific CD8+ CTL are the most important defense mechanism against outgrowth of EBV infected B cells. These cells recognize peptide fragments derived from viral antigens expressed on the surface of B cells in association with MHC. Studies using EBV latent proteins expressed in vaccinia virus constructs have shown that a number of antigens can serve as targets (19,20).

The importance of cytotoxic T cells is illustrated by patients with the rare familial disorder X-linked lymphoproliferative syndrome. These patients have an abnormal response to EBV and present in the first decade of life with

either fulminant mononucleosis or EBV lymphoproliferation (21). In addition patients with acquired defects in T lymphocyte function such as transplant recipients or patients with AIDS have an increased risk of EBV lymphoproliferation.

3.6 Immune Reconstitution Post MUD or Mismatched Family Donor BMT

Conditioning regimens for BMT destroy the recipient's own immune and hemopoietic systems. Recovery is dependent on the establishment of a graft and the development of donor-derived hemopoietic and immune systems. After T cell-depleted matched sibling allograft or autograft there is rapid recovery of activated CD3+CD16- and CD16+CD3- activated killer cells which are cytotoxic towards EBV infected targets and spontaneously secrete cytotoxic cytokines (22,23). The generation of these endogenously activated cells may help explain the low incidence of EBV after MHC identical sibling grafts. Immune recovery post MUD transplant is less well characterized. In preliminary studies at the Royal Free Hospital in patients receiving T cell depleted MUD BMT, activated killer cells were not found in the early post transplant period (H Heslop, D Gottlieb unpublished observations). In addition recovery of CD3+ cells was delayed. Studies in patients receiving MUD or mismatched family BMT at St Jude confirm that recovery of CD3+ T cells is slower than in patients receiving matched sibling BMT. Although CD3 numbers are low studies of repertoire usage do not show any specific defects (24).

3.7 Possibility of Generating Immune System Effector Cells to EBV

One approach which may improve immune defenses against viruses following matched unrelated or mismatched BMT is to adoptively transfer donor derived virus specific cytotoxic T lymphocytes. This strategy is successful in animal models and is currently being explored in human BMT recipients at the Fred Hutchinson Cancer Center in Seattle using CMV as a model (25). These investigators had previously documented that the risk of CMV disease correlated with the generation of spontaneous CMV specific cytotoxic T lymphocytes post transplant. To extend this observation they generated CMV specific cytotoxic T lymphocytes from BMT donors in vitro and in a dose escalation study adoptively transferred these virus specific cells to recipients. Administration of such cells to 5 patients resulted in no clinical adverse events and detection of CMV specific responses.

In this study we wish to generate cytotoxic T cells specific for EBV and adoptively transfer these cells to recipients at high risk for EBV reactivation and lymphoproliferation. Our aim is to track the infused cells by means of a marker gene, analyze their activity after infusion, and determine a safe and efficacious cell dose for subsequent analysis as prophylaxis for EBV disease. We have already generated EBV specific cytotoxic T cells from one donor

whose recipient developed an EBV lymphoproliferation after a 2 antigen mismatched transplant. These cells were generated in Dr Rooney's laboratory by coculturing donor peripheral blood lymphocytes with irradiated donor lymphoblastoid cell line. The line was expanded by addition of IL2 and weekly restimulation with irradiated autologous lymphoblastoid cells. Phenotyping showed the line was CD3+ CD8+ and cytotoxicity assays showed the line killed autologous LCLs but not allogeneic LCLs. Killing could be blocked by antibody to Class I MHC antigens.

As the recipient had progressive EBV lymphoproliferation despite therapy with antibodies to CD21 and CD24 and Interleukin 2, we obtained IRB emergency approval to administer EBV specific cytotoxic T cells. At the time of infusion she had pulmonary and renal nodules, paratracheal nodes resulting in respiratory compromise and bowel infiltration resulting in gastro-intestinal bleeding. She received 3×10^7 cells/m² with no adverse effects; in particular she did not develop GVHD. However her paratracheal nodes continued to grow and 4 days later she required local irradiation and steroid therapy. At this stage her parents wished her to have no further therapy and she was discharged home where she died. Autopsy showed bowel hemorrhage and perforation was the cause of death but also showed necrosis of her pulmonary and renal nodules. While any beneficial effects on the pulmonary and renal nodules cannot confidently be ascribed to the CTL, she did not experience any adverse effects from the infusion. These results offer further encouragement to the proposed prophylaxis safety study.

3.8 Risks of Administering EBV Specific CTL

The main risk of this adoptive transfer approach would be that the donor derived anti-EBV T cells would cross react with host alloantigens and cause GVHD. Such reactivity would be unlikely due to the method of generation of the CTLs, and we will screen the CTL lines to ensure specificity for EBV infected targets. No GVHD has been observed in the Seattle patients receiving cytotoxic T lymphocytes (25) nor in the St Jude patient detailed above. Although we will screen CTL lines for reactivity against other host tissues such as bone marrow fibroblasts and PHA blasts, there is no completely reliable in vitro assay for excluding this possibility. We will take several additional precautions to minimize this risk. First we will not administer CTLs until Day +45 when the highest risk for GVHD has passed. Any patient with GVHD of greater than Grade 1 will be excluded from the study at this point. Second we will administer CTLs in escalating doses starting at 10^7 cells/m². This is a much smaller number of T cells than administered at the time of marrow infusion even with a T cell depleted marrow. Finally, should any patient develop GVHD the site will be biopsied and examined histologically to confirm the clinical diagnosis. In addition DNA will be extracted and examined for the neomycin resistance gene (see below) to determine if administered CTL were present in the biopsy.

A second potential hazard is the infusion EBV transformed B cells which have been cocultured with the CTL during generation of the T cell lines. This is unlikely to constitute an additional risk to the recipient for several reasons. First the lymphoblastoid cells are not viable because they have been irradiated with 4000cGy and cocultured with known effectors. Second the lymphoblastoid cells are donor type and will be spontaneous transformants; the recipient has already received unirradiated donor B cells containing the same strain of latent virus with the marrow graft. Thirdly we will add Acyclovir to the T cell line cultures (Appendix 1) so no productive virus will be present in cultures. To confirm that no productive virus is present the ability to transform cord blood will be tested. Finally we will monitor levels of EBV DNA in peripheral blood by PCR pre and during CTL infusions.

3.9 Availability of Gene Marking

In June 1991, St. Jude Children's Research Hospital was the first center outside the NIH to receive RAC/FDA approval for human gene transfer (26-28). Amongst the protocols approved was the insertion of a selectable marker gene (neomycin resistance) in the LNL6 vector into the marrow of children with AML in remission, neuroblastoma in remission or advanced neuroblastoma. This marrow is reinfused as an autologous bone marrow transplant (ABMT). The primary aim is to determine whether AML or neuroblastoma cells at relapse are marked, since 50-70% of the children will likely relapse after treatment on these frontline studies. As of November 1992, sixteen patients have received gene marked marrow. Two patients with AML have relapsed and in both cases the neomycin resistance gene has been detected in leukemic cells at the time of relapse showing that leukemic cells in so called remission marrow can contribute to relapse (29). In addition the study has provided information about hemopoietic reconstitution and expression of leukemic vectors as the neomycin resistance gene has been detected in hemopoietic cells and T lymphocytes for up to a year post autograft (30).

The expertise in the BMT laboratory in this area thus affords an opportunity to monitor the survival and recirculation of EBV specific cytotoxic T lymphocytes administered to BMT recipients. Should the patient develop GVHD, we will analyze part of the routine tissue biopsy by PCR to determine if gene marked T cells are present.

4.0 STUDY DESIGN

4.1 EBV Lymphoblastoid Cell Line Preparation

At the time of final donor-recipient compatibility typing by MLC, 5cc extra blood will be requested through Dr Turner's laboratory and given to Dr Recombinant DNA Research, Volume 17

Rooney to establish donor derived lymphoblastic cell lines. We will attempt to establish spontaneous EBV infected LCLs as described in Appendix I. These lines will take about a month to establish and will be expanded to 10^8 cells and frozen.

4.2 Cytotoxic T Cell Line Preparation

At the time of pretransplant evaluation for MISMUD the ETNA protocol will be discussed with the patients and parents and consent obtained. Ten cc of peripheral blood will be obtained from the donor at the time of bone marrow harvest and used to generate EBV specific CTLs as described in Appendix I. After establishment the CTL lines will be checked for specificity and microbiological culture.

4.3 Transduction With the Neomycin Resistance Gene

CTL are transduced with the LNL6 or G1Na vectors used in the previous St. Jude gene marking protocols, AMLREM, NEBREL and NEBREM. The efficiency of marking will be estimated by clonogenic growth in G418 and semi-quantitative PCR for the neomycin resistance gene.

Both LNL6 and G1Na have been extensively studied in rodent models and in non-human-primates and in man since 1989. The results of the first *in vivo* human studies on transduced cells have been reported, as have our own safety data using marrow cells as the vector target (29,30,31). The safety data for LNL-6 and G1Na are contained within the Drug Master Files # BB-MF-3886 and BB-MF-4195 which were used for granting our institutional IND's 3838 (LNL6) and 4272 (G1N). The current proposal will cross reference these data. The major safety concerns and their resolution have been dealt with at length in previous published protocols.

4.4 Administration and Monitoring

At Day 45 patients will be evaluated for eligibility by one of the principal investigators and consent obtained. CTLs will be administered weekly in escalating doses. Patients will be monitored for clinical toxicity by standard NIH criteria (Appendix 2) and for GVHD by the Seattle criteria (Appendix 3). In addition we will monitor the kinetics of CTL survival by monitoring the presence of the marker gene in peripheral blood. We will also analyze immunological effects by monitoring lymphocyte phenotype, changes in repertoire of T cell receptors, and cytotoxic effector function in the BMT laboratory. Dr Rooney's laboratory will monitor the levels of EBV DNA in peripheral blood before and following infusion.

5.0 PATIENT ELIGIBILITY (See Section 1.0)

5.1 All patients enrolled on the MISMUD protocol will be eligible for this protocol.

5.1.1 Patients with ALL or high grade (Stage III or IV) T cell non-Hodgkin lymphoma who obtain a clinical remission after having relapsed during or after treatment with intensive multiagent chemotherapy.

5.1.2 Patients with a proven diagnosis of Ph⁺ ALL in first or subsequent clinical remission.

5.1.3 Patients with CML in chronic or accelerated phase.

5.1.4 Patients with high risk AML (Monosomy 7, secondary AML, 5q-) in first clinical remission.

5.1.5 Patients with JCML or a myelodysplastic syndrome who have not transformed to a frank AML.

5.1.6 Patients who have active leukemia treated as per the MISMUD protocol.

5.2 Exclusion Criteria

5.2.1 Exclusion criteria for mismatched or unrelated donor BMT will be as detailed in the MISMUD protocol, except patients with active leukemia at the time of BMT will be eligible for ETNA.

5.2.2 Exclusion criteria at time of administration CTLs.

5.2.2.1 GVHD of Grade II or greater.

5.2.2.2 Patients with severe renal disease (i.e., creatinine clearance less than half normal for age).

5.2.2.3 Patients with severe hepatic disease (bilirubin greater than twice normal, or SGPT greater than 3 x normal).

5.2.2.4 Patients with a severe intercurrent infection.

5.2.2.5 Life expectancy <6 weeks.

5.2.2.6 Patients must have normal nutritional status (weight >10th percentile for age, serum albumin >3 gm/dl) and Performance Status (ECOG) of 0-2.

Performance Status Definition

<u>ECOG Grade</u>	<u>Karnofsky Score</u>	<u>Activity</u>
0	90-100	Up and about, no restriction.
1	70-90	Ambulatory, no strenuous activity.
2	50-70	Ambulatory, capable of self-care appropriate for age. Up and about >50% of time, but unable to carry out any physical activities or attend school.
3	30-50	Limited self-care only. Up and about <50% of time.
4	<30	Disabled, no self care. Bedridden or confined to chair.

6.0 TREATMENT PLAN

6.1 Cell Administration

Cell administration will be as follows:

EBV specific gene-marked T cells will be given by intravenous injection in RPMI with 5% HSA at weekly intervals x 4 from Day 45 at the following dose schedules. Three to six patients will be treated in each group (See Section 9).

Group One

Day 45	10^7 cells/m ²
Day 52	10^7 cells/m ²
Day 59	5×10^7 cells/m ²
Day 66	5×10^7 cells/m ²

Group Two

Day 45	5×10^7 cells/m ²
Day 52	5×10^7 cells/m ²
Day 59	10^8 cells/m ²
Day 66	10^8 cells/m ²

Group Three

Day 45	10^8 cells/m ²
Day 52	10^8 cells/m ²
Day 59	5×10^8 cells/m ²

Day 66 5×10^8 cells/m²
Cells will be resuspended in a volume of 5-20ml.

6.2 Patients will be premedicated with Benadryl 1mg/kg IV and Tylenol 10mg/kg PO

6.3 Patients may receive CTLs in the medicine room if they are an outpatient.

Monitoring will be undertaken according to institutional standards for administration of blood products with the exception that the injection will be given by a physician. Antiemetics in appropriate dosage for each patient will be prescribed as necessary.

6.4 Patients will receive supportive care for acute or chronic toxicity, including blood components or antibiotics, and other intervention as appropriate.

6.5 All treatments will be given at St. Jude Childrens' Research Hospital.

7.0 PATIENT EVALUATION

7.1 A complete history and physical examination is necessary prior to administration of CTLs on Day 45.

7.2 Other Studies

7.2.1 The following investigations will be obtained pre infusion, then 3 x weekly for 6 weeks, then weekly for 6 weeks then monthly for 1 year.

CBC and diff

Urinalysis

BUN, creatinine, bilirubin, SGOT, SGPT, alkaline phosphatase, Na, K, Cl, CO₂, albumin, total protein, glucose.

7.2.2 The following investigations will be obtained pre infusion, then weekly for 6 weeks, monthly for 1 year and yearly for 10 years.

Peripheral Blood in preservative free heparin (20 ml for BMT lab). This blood will be used for the following immunological and marker gene studies.

Immunophenotyping

Cytotoxicity assays

T cell receptor repertoire analysis

EBV DNA content

Analysis for the marker gene by PCR and reverse transcriptase assay will be done for 10 years.

7.3. Other tissues

- 7.3.1 Punch biopsies of appropriate skin sites, GI tract or liver biopsies will be done if the patient should develop GVHD.

8.0 EVALUATION DURING STUDY

8.1 Follow-up Interval

Patients shall be seen at thrice weekly intervals at St. Jude Children's Research Hospital for 6 weeks, then weekly for 6 weeks then monthly for one year. Additional visits will be obtained as clinically indicated.

8.2 Early Termination of Study and Modifications of Drug Dosages

- 8.2.1 This study will be complete when 3-6 patients have been entered at each dose level (See Section 9).

- 8.2.2. Therapy for an individual patient can be terminated if a dose limiting toxicity has been observed.

- 8.2.3 If the patient/parent desires to withdraw from the study, or if the physician feels that it is in the patient's best interest, treatment will be discontinued.

- 8.2.4 If Grade 3/4 toxicity is encountered in these studies, the Institutional Review Board will be notified and dosage modifications made before admission of additional patients to confirm the maximum tolerated dose.

- 8.3 The criteria listed in Appendix II will be used in grading toxicity.

- 8.4 For consistency, toxicity for all patients will be quantitated using these criteria at the end of the trial.

- 8.5 A 4 week period will constitute a course which will be evaluated for critical toxicity, and a 6 week period will be required for evaluation for antitumor activity and for late toxicity.

9.0 STATISTICAL CONSIDERATIONS

9.1 Design (stopping rule)

When three of the first three (or five of the first six) patients have successfully completed a dose level without evidence of dose limiting toxicity (DLT)

subsequent patients may be entered at the next dose level. DLT is defined as NIH Grade III-IV toxicity (Appendix II) in any system that can be attributed to treatment or Grade III-IV GVHD (Appendix III) with Neo^R gene containing T cells detected on biopsy.

If DLT occurs in 1 of 3 patients at a given dose, an additional 3 patients will be evaluated at that level. If none (0) of these 3 patients experience DLT, the TSE is escalated for the next cohort of 3 patients. However, if DLT occurs in 1 of the additional 3 patients, then the MTD has been exceeded and 3 more patients are treated at the previous lower dose level if only 3 patients were treated that level.

If DLT is noted in 2 of 3 patients at a given dose level, the MTD has been exceeded and 3 more patients are treated at the next lower level (if only 3 patients were treated previously at that dose).

At any time that 2 patients in a dose cohort (of 3-6) patients experience DLT, no further patients will be entered at that dose and the study will be halted for modification as considered appropriate by local and federal regulatory authorities.

The MTD is defined as that dose level immediately below the dose level at which 2 or more patients of a cohort (of 3-6 patients) experience dose limiting toxicity.

9.2 Number of Subjects

Because of the Phase I design, the exact number of patients required to complete this trial cannot be determined. At least three dose levels are planned and 3-6 patients will be treated per level. We anticipate enrolling approximately 10-15 patients over a period of 18-24 months.

9.3 Design Parameters

With the escalation plan outlined in section 9.1, the probability of observing 2 patients with toxicity at a given dose level (conditional on starting a level) is 9%, 51% and 83% if the true incidence of toxicity associated with that dose is 10%, 30% or 50%, respectfully.

The following table gives estimates of 95% confidence intervals of the true incidence toxicity based on the observed incidence and the sequential nature of the stopping rule. This table simply emphasizes the lack of precision of the phase I design for estimating the true incidence of DLT.

Observed Outcome	Estimated Incidence	Exact 95% Confidence Interval
MTD (0/3)	0 %	0 - 63 %
MTD (1/6)	17 %	0 - 66 %
DLT (2/4)	33 %	2 - 70 %
DLT (2/5)	40 %	3 - 77 %
DLT (2/4)	50 %	6 - 89 %
DLT (2/3)	67 %	10 - 99 %
DLT (2/2)	100 %	22 - 100 %

10.0 STUDY INTERPRETATION

Since this is a Phase I study the main aim will be to collect information about dosage and immunomodulatory and virological efficacy for a future Phase II study. Potential problems in this preliminary Phase I study would include toxicity with development of GVHD or lack of efficacy as evidenced by absence of immunomodulatory effects.

10.1 Excessive Toxicity

If administration of CTL was associated with an unacceptably high risk of GVHD we would modify the study in two ways. First we would clone the CTLs and administering only clones that lysed EBV infected cells but not other allogeneic or autologous cells. Second we would transduce the CTLs with the Tk vector, so that the CTLs may be killed by administration of Acyclovir should toxicity occur. This approach presents some logistic difficulties as all CMV+ recipients are at present on Ganciclovir prophylaxis for CMV. However in the next year we plan to start a trial of Foscarnet prophylaxis for CMV so this approach would be possible if required.

10.2 Lack of Efficacy

If patients on the lower dose schedules developed EBV lymphoproliferation, this would not necessarily be regarded as a failure as outgrowth of EBV infected B cells may reflect insufficient numbers of adoptively transferred CTLs. If however patients receiving higher doses developed lymphoproliferation or failed to show immunomodulatory effects we would aim to enhance the activity of the transferred CTLs. Possible approaches would include transducing the CTLs with cytokine genes such as IL1 or cytokine receptor genes. Such manipulations have

enhanced cytotoxic effector function in animal models. Again such constructs would need to include the Tk suicide gene.

11.0 OFF-STUDY CRITERIA

- 11.1 Any patient who develops irreversible life-threatening non-hematologic organ system dysfunction (toxicity grade 3 or 4) considered to be primarily related to CTL injection will be removed from this protocol and will not be eligible for subsequent treatment with CTLs.
- 11.2 Any questions regarding patients on this study should be addressed to Dr. Heslop (Ext 2529) or Dr. Brenner (Ext 410).

12.0 RECORDS TO BE KEPT

Flow sheets documenting dates and doses of therapy as well as clinical chemistries, hematologic parameters, the clinical status and occurrence of any adverse events and subsequent interventions are to be kept on all patients.

Imaging reports

Surgical summaries

Autopsy summaries, where appropriate

Date of entry on to other appended protocols

Informed consent documents

All required clinical evaluation records will be the responsibility of Dr. Helen Heslop who will also be responsible for analysis of the clinical outcome and toxicity.

The laboratory evaluation of immunological efficacy and gene insertion will be the responsibility of Drs. Heslop and Brenner. The laboratory evaluation of EBV DNA persistence will be the responsibility of Dr Rooney.

13.0 REPORTING REQUIREMENTS

- 13.1 Register all patients with Bone Marrow Transplant Data Manager at Ext 410.

13.2 Enter all patients by phoning Dr. Heslop or Dr. Brenner. The following forms should be completed:

On study form

Investigational agents flow sheet forms

Off study forms

13.3 Adverse reactions will be reported by phone to Dr. Heslop or Dr. Brenner within 24 hours of their occurrence. These adverse reaction reports include all life-threatening events (Grade 3/4 toxicity, Section 9.0) which may be due to tumor cell injection, and all fatal events.

14.0 INFORMED CONSENT

All patients and/or their legal guardian must sign a document of informed consent consistent with local institutional and Federal guidelines stating that they are aware of the investigational nature of this protocol and of the possible side effects of treatment. Further, patients must be informed that no efficacy of this therapy is guaranteed, and that unforeseen toxicities may occur. Patients have the right to withdraw from this protocol at any time. No patient will be accepted for treatment without such a document signed by him or his legal guardian. Full confidentiality of patients and patient records will be provided according to institutional guidelines.

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16.0 PARENT'S/PATIENT'S SUMMARY STATEMENT

ADMINISTRATION OF NEOMYCIN RESISTANCE GENE MARKED EBV SPECIFIC CYTOTOXIC T LYMPHOCYTES TO RECIPIENTS OF MISMATCHED-RELATED OR PHENOTYPICALLY SIMILAR UNRELATED DONOR MARROW GRAFTS FOR LEUKEMIA

You have/your child has a type of blood cell cancer that is very hard to cure with standard treatments and you/your child will receive a bone marrow transplant. Because you/your child does not have a brother or sister whose marrow is a "perfect match", this bone marrow will come from a donor whose marrow is the best match available. This may be a close relative or an unrelated person whose bone marrow best "matches" yours/your child's, and who agrees to donate marrow. As this marrow will not be as closely matched as a brother or sister who is a "perfect match" there is a higher risk of problems. One of these problems is that because the new immune system may be slow to grow back you/your child will be at risk of serious or fatal viral infections.

EPSTEIN-BARR VIRUS (EBV) INFECTION

One virus that can cause problems after this type of transplant is Epstein Barr virus (EBV). In normal people, this virus causes a flu like illness (sometimes called infectious mononucleosis or glandular fever or kissing disease) and usually gets better when the immune system controls the infection. However the virus remains hidden in the body for life. After a transplant, while the new immune system is growing back, the EB virus can come out and infect cells and cause them to grow in an uncontrolled manner. Patients can develop fevers, swollen lymph nodes and damage to other organs such as kidneys and lungs. This infection acts like a cancer because the cells infected with EB virus grow very quickly and there is no known effective treatment. This sort of infection will occur in between 10-30% of patients receiving a transplant from a donor who is not a perfect match and is fatal in nearly all these cases.

This infection occurs because the immune system cannot control the growth of the cells. We want to see if we can prevent it from happening by giving you/your child special T cells that we have grown from your/your child's marrow donor. These cells have been trained to attack EB virus infected cells. We will grow these T cells from blood taken from your/your child's donor at the time of bone marrow harvest. These T cells will be stimulated with the donor's EB virus-infected cells which have been treated with radiation so they cannot grow. After mixing these cells together we will be able to grow special T cells from the donor that can attack EBV infected cells. We will then collect the T cells and make sure they can kill the virus infected cells.

MARKING OF T CELLS

To learn how long these T cells last when we have given them, we will mark them with a special bacterial marker gene. We will use a mouse virus that has been changed to keep it from causing infection. The marker, a gene called Neo^R is put inside this special virus.

INJECTION OF CELLS

The cells will then be injected into the central line over 10 minutes with a premed. We would give four doses every week starting at Day 45 after the transplant if you/your child agreed and were well enough.

BLOOD TESTS AFTER THE TRANSPLANT

To learn more about the way the T cells are working and how long they are lasting, an extra 10-20 mls (about 2 tablespoons) of blood will be taken every week for six weeks after the T cell infusions, and then every month for one year. It should come from the central line, and should not require extra needle sticks.

CONFIDENTIALITY

Your/your child's case will be treated in complete confidence, unless you specifically agree otherwise. However, approved people from the Food and Drug Administration and National Institutes of Health, as well as St. Jude clinical staff, may review the charts.

RISKS AND SIDE EFFECTS

These types of T cells have been given to animals and to some patients with no side effects. However there is a possibility that these T cells might try to attack other parts of your/your child's body and cause graft versus host disease (GVHD). GVHD occurs when cells from your/your child's bone marrow donor (graft) recognizes that your/your child's body tissues (host) are different from those of the donor. When this happens cells in the graft may attack the host's skin, liver and intestines.

If you/your child has GVHD after the transplant you will not be able to get the T Cells. If you/your child gets GVHD after the T cells have been given we will not give you any more of them. We will treat you/your child with steroids, and if necessary, an antibody to kill the T cells. There may also be a risk from exposure to donor EB virus but this will be less than with the infusion of the donor's bone marrow.

No bad effects at all have been seen in animal studies using the gene markers or in more than 30 people treated with marker genes. Still there may be some risks. It is possible the mouse virus may be able to recover in the cell; it may even be able to cause cancer. We think this is very unlikely but cannot rule it out yet.

BENEFITS

We hope that these cells will reduce the risk of you/your child developing a serious or fatal infection with EB virus and that the marker genes will allow us to see how the cells are working.

ALTERNATIVE TREATMENT

You/your child may have the transplant without receiving the cytotoxic T lymphocytes.

The table below lists the short- and long-term side effects of this treatment.

= rare side effects

<u>Agent</u>	<u>Possible side effects</u>
Infusion of T cells	Graft versus host disease Allergic reaction Skin rash Trouble breathing
Marker gene (vector)	No known side effects (Possibly could cause some antibiotics such as neomycin and amikacin not to work or could regrow or could make cells cancerous)
Prednisone	Increase in appetite Weight gain Higher risk of infection High blood sugar (temporary diabetes) Acne Stomach ulcers with bleeding Changes in mood or behavior Decreased growth (shorter than expected) if given over long periods Bloating (retaining fluids)

17.0 STATEMENT OF UNDERSTANDING

1. I have read the above statement and voluntarily agree to the participation of myself/my child _____ in this study/treatment.
2. I have had adequate opportunity to discuss with Dr. _____ caring
(name of investigator)
for me/my child _____ all the purposes and hazards related to this study/treatment.
3. I have been told of alternate courses of action in my/my child's situation.
4. I understand that the study/treatment proposed may result in risks which are currently unforeseeable.
5. I understand that I may withdraw myself/my child from this study/treatment at any time without jeopardy to further treatment.
6. I understand that financial compensation is not provided for participation in this study/treatment.
7. I understand that in the event of non-negligent physical injury to myself/my child from this study/treatment, financial compensation is not available but medical treatment necessary will be provided at no direct cost.
8. I understand that the information obtained will be kept confidential but that supervised review by approved medical representatives of outside institutions or agencies may occur. In particular, the chart may be reviewed by an agent of the Food and Drug Administration or National Cancer Institute.
9. I understand that if I have further questions regarding this study/treatment, or concern about injury from this study/treatment, I can contact Drs M.K. Brenner or H Heslop at 901 522 0300
10. I understand that further information regarding my/my child's rights as a research participant can be obtained by contacting the Chairman of the Clinical Trials Committee, at 901/522-0300.
11. I understand that I shall receive a signed copy of this summary statement.

Patient

Parent

Physician

Witness

Date

Time

In case of questions or emergencies in reference to this protocol, please contact:

St. Jude Children's Research Hospital
332 North Lauderdale
Memphis, TN 38105
Telephone: (901) 522-0300

ASSENT FORM - PATIENT AGE 7-18 YEARS FOR CTL THERAPY.

ASSENT

You have a type of blood cell cancer that is very hard to cure with standard treatments and you/your child will receive a bone marrow transplant. Because you do not have a brother or sister whose marrow is a "perfect match", this bone marrow will come from a donor whose marrow is the best match available. As this marrow will not be as closely matched as a brother or sister who is a "perfect match" there is a higher risk of problems. One of these problems is that because the new immune system may be slow to grow back you will be at risk of serious or fatal viral infections.

One virus that can cause problems after this type of transplant is Epstein Barr virus (EB virus). In normal people, this virus causes a flu like illness (sometimes called infectious mononucleosis) and usually gets better when the immune system controls the infection. However the virus remains hidden in the body. After a transplant, while the new immune system is growing back, the EB virus can come out and infect cells and cause them to grow in an uncontrolled manner.

This infection occurs because the immune system cannot control the growth of the cells. We want to see if we can prevent it from happening by giving you special T cells that we have grown from the marrow donor. These cells have been trained to attack EB virus infected cells. The cells will be injected into the central line over 10 minutes with a premed. We would give four doses every week starting at Day 45 after the transplant if you agreed and were well enough.

To learn how long these T cells last when we have given them, we will mark them with a special virus. This special virus is like a taxicab and drops off a marker in your cells.

To learn more about the way the T cells are working and how long they are lasting, an extra 10-20 mls (about 2 tablespoons) of blood will be taken every week for six weeks after the T cell infusions, and then every month for one year. It should come from the central line, and should not require extra needle sticks.

SIDE EFFECTS

These types of cells have been given to animals and to some patients with no side effects. However there is a possibility that these T cells might try to attack other parts of your/your child's body and cause graft versus host disease (GVHD). GVHD occurs when cells from your/your child's bone marrow donor like the marrow graft or these T cells (graft) recognizes that the body tissues of the patient (host) are different from those of the donor. When this happens cells in the graft may attack the host skin, liver and the gut.

If you/your child have GVHD after the transplant you/your child will not be able to get the T cells. If you/your child get GVHD after the T cells have been given we will not give you/your child any more of these. We will treat you/your child with steroids and if necessary an antibody to kill the T cells.

No bad effects have been seen in animal studies using the special virus markers or in more than 30 people treated with these markers, but it is possible that the special virus may grow or stop some antibiotics from working or even cause cancer. We do not think this will happen, but it is possible.

ALTERNATIVE PROCEDURES

You/your child can have no extra treatment at all, but we believe that this extra treatment will reduce your risk of infection with EBV after your/your child's transplant.

STATEMENT OF UNDERSTANDING

All information about your/your child's treatment will be kept among the hospital staff that needs to know. We will not tell anybody else without asking you first. You/your child have the right to ask any questions you/your child want and they will be answered. You/your child have had this assent form read or discussed with you by your parents and the doctor (Dr. _____) caring for you/your child. Before you signed this form you had all your questions answered.

If you have any other questions you can ask your doctor or Dr. Helen Heslop or Dr. Malcolm Brenner at any time.

Patient

Parent or Guardian

Physician

Date and Time

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

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INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
University of Otago, Dunedin, New Zealand	MB ChB	1980	Medicine
Royal Australasian College of Physicians	FRACP	1987	Medicine
Royal Australasian College of Pathologists	FRCPA	1987	Hemat/Immunol
University of Otago, Dunedin, New Zealand	MD	1990	Hematology

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

1980-82 House Officer/Senior House Officer, Canterbury Hospital Board, Christchurch, New Zealand

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1989-91 Postdoctoral Research Associate, Department of Biochemistry and Hematology-Oncology, St. Jude Children's Research Hospital, Memphis, TN

1991 to present Assistant Professor, Department of Pediatrics, University of Tennessee, Memphis, College of Medicine, Tennessee

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PUBLICATIONS (From a total of 46)

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- Heslop HE, Krance RA, Parham D, Brenner MK. Viruses and transplantation. *Encyclopedia of Virology* (in press).

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

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RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

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 1990 to present Member, Hematology/Oncology, and Director, Bone Marrow Transplant Program, St. Jude Children's Research Hospital, Memphis, Tennessee

SELECTED PUBLICATIONS (of 137 total)

Wimperis JZ, Brenner MK, Prentice HG, Reittie JE, Patterson J, Karayiannis P, Griffiths PD, Hoffbrand AV. Transfer of a functioning humoral immune system in transplantation of T lymphocyte depleted bone marrow. Lancet i:339-343, 1986.
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Duncombe AS, Grundy JE, Oblakowski P, Prentice HG, Gottlieb DJ, Roy DM, Reittie JE, Bello-Fernandez C, Hoffbrand AV, Brenner MK. Bone marrow transplant recipients have defective MHC unrestricted cytotoxic responses against cytomegalovirus in comparison with Epstein-Barr virus: the importance of target cell expression of lymphocyte-function-associated antigen (LFA1). *Blood* 79:3059-3066, 1992.

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CONTINUATION PAGE: STAY WITHIN MARGINS INDICATED

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

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University of East Anglia, England	B.Sc.	1978	Genetics
University of Cambridge, England	Ph.D.	1981	Immunology

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

- 1981-84 Postdoctoral Fellow, (Department) University of Bristol, England
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 1987-89 Assistant Member, Ludwig Institute for Cancer Research, St. Mary's Hospital Medical School, London, England
 1990 to Present Assistant Member, Department of Virology and Molecular Biology, St. Jude Children's Research Hospital, Memphis, Tennessee

PUBLICATIONS

Rooney CM, Rickinson AB, Moss DJ, Lenoir GM, Epstein MA. Paired Epstein-Barr virus-carrying lymphoma and lymphoblastoid cell lines from Burkitt's lymphoma patients: Comparative sensitivity to non-specific and to allospecific cytotoxic responses *in vitro*. Int J Cancer 34:339-348, 1984.

Rooney CM, Rowe M, Wallace LE, Rickinson AB. Epstein-Barr virus positive Burkitt's lymphoma cells not recognized by virus-specific T-cell surveillance. Nature 317:629-631, 1985.

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Rooney CM, Gregory CD, Rowe M, Finerty S, Edwards C, Rupani H, Rickinson AB. Endemic Burkitt's lymphoma: Phenotypic analysis of tumor biopsy cells and of the derived tumour cell lines. J Natl Cancer Inst 77:681-687, 1986.

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HUMAN GENE TRANSFER PROTOCOL

9303-039 Brenner, Malcolm K.; Krance, Robert; Heslop, Helen E.; Santana, Victor; and Ihle, James, St. Jude Children's Research Hospital, Memphis, Tennessee; *Assessment of the Efficacy of Purging by Using Gene-Marked Autologous Marrow Transplantation for Children with Acute Myelogenous Leukemia in First Complete Remission.*

Date of RAC Approval: 3/2/1993

Date of NIH Approval: 4/16/1993

SUMMARY

Autologous bone marrow transplantation (ABMT) may be a useful way of permitting an increased intensity of treatment of patients with acute myeloid leukemia (AML), potentially curing a higher proportion of patients. However, the marrow harvested in apparent clinical remission may contain residual malignant cells which contribute to disease recurrence. We have been using the Neo^R containing retrovirus vectors LNL6 and G1N to mark of one-third of all harvested autologous marrow to trace the origin of relapse.

To date, 11 patients with AML have received gene marked marrow. Marking did not delay engraftment compared to historical controls. Two patients have subsequently relapsed. In both, a proportion of the relapsed cells contained the marker gene. Since this study shows that "remission" marrow may indeed be contaminated with malignant cells that can contribute to relapse, we now propose to incorporate marrow purging into the protocol, in an attempt to remove these cells.

We intend to use both the LNL6 and G1N vectors in every patient who will receive an ABMT. Each vector will be used to mark one-third of the marrow. The remaining third will be cryopreserved unmanipulated as a back up. Each of the marked portions of marrow will be randomized to be purged either with 4-hydroxyperoxycyclophosphamide or with interleukin-2. After purging the marrows will be reinfused. If the patients relapse, we will discover if the marker is present in the relapse cells, and if it is, with which purging technique the relapse is associated. In this study we propose to treat 35 patients.

NON-TECHNICAL SUMMARY

Autologous bone marrow transplantation is a useful way of treating many cancers, including acute myeloid leukemia. Because patients still relapse after the procedure, there has been concern that the marrow that is used for the autologous transplant is contaminated with malignant cells which simply re-introduce the disease. Our early studies using retroviral gene marking of the marrow before re-infusion it has allowed us to trace the origin of relapse.

In two patients whose disease has recurred after autologous BMT, the marker gene was indeed found in the malignant cells. This demonstrates that giving back patients' own marrow in this disease may also give them back their leukemia.

In the current proposal we will try to remove these malignant cells by marrow purging. First we will mark one third portions of the marrow with the distinguishable retroviruses LNL6 and G1N. One third will be cryopreserved as a safety back up. Each of the marked portions of marrow will be treated with a purging drug, either 4HC or IL2. If the patients relapse, we will be able to see whether marked cells were still present, despite purging, and see whether any one method of purging was more effective. We propose to treat 35 patients.

SJCRH
PAMMYLA
12/92

ASSESSMENT OF THE EFFICACY OF PURGING BY USING
GENE MARKED AUTOLOGOUS MARROW TRANSPLANTATION FOR
CHILDREN WITH AML IN FIRST COMPLETE REMISSION:

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Helen E. Heslop MD
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James Ihle, Ph.D.

CO-INVESTIGATORS

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W. Mark Roberts, M.D.
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1.0 OBJECTIVES

- 1.1 To use transduction of both the GIN and LNL6 marker retroviruses into separate portions of each patient's autologous marrow to determine:
 - a) whether purging eliminates gene marked malignant cells from the harvested marrow, so that marker genes are absent in the malignant population at the time of relapse.
 - b) whether marrow purging with 4HC or with IL2 is more effective at preventing relapse with gene marked malignant cells.
 - c) how each method of purging modifies the pattern of autologous reconstitution.
- 1.2 To estimate the continuous complete remission rate at 2 years for children with AML in first complete remission treated with 4HC and IL2 purged Autologous Bone Marrow Transplant (ABMT).

2.0 BACKGROUND

2.1 Current Status of Therapy for Acute Nonlymphocytic Leukemia

Despite improvements in chemotherapy regimens and supportive care, 15% to 20% of children with acute myeloid leukemia (AML) fail to achieve complete remission (CR) (1-4). Further, most children who achieve CR then relapse and in most studies the median duration of complete continuous remission (CCR) rarely exceeds 18 months. Long-term survival is realized in 20-40% of patients treated with current cytotoxic drug therapy. Reinduction chemotherapy regimens have limited success; brief second CRs are achieved in only 40% to 50% of patients (1-4).

The major therapeutic problem in AML is effectively eliminating the residual leukemic cells after patients achieve a complete remission. Explanations proposed for treatment failure include: (1) variations in patients' systemic drug metabolism leading to insufficient exposure of leukemic cells to the active drug; (2) variations in leukemic cellular metabolism of drugs leading to insufficient leukemic cell kill; (3) the development of multidrug resistance, either classical (mediated by the MDR1 gene product) or "atypical" in nature; (4) inability of maintenance therapy to eliminate the residual leukemic cell burden due to failure of the clonogenic leukemia cells to enter the cell cycle, thus precluding cytotoxic effects of S-phase-specific drugs (1-4).

Bone marrow transplantation has been used to increase the long-term survival for patients with acute leukemia and holds the major hope for cure in patients who relapse (5-12). For patients undergoing an HLA-matched sibling allogeneic bone marrow transplant (AlloBMT), the long-term survival is approximately 50% for AML transplanted in first remission (10). Unfortunately, only 30% of patients with acute leukemia have a HLA-matched donor (9-13).

In patients who survive alloBMT and do not relapse, there is a significant risk of chronic graft-versus-host disease (GVHD). The success of AlloBMT is related to the patients age; pediatric patients have a better outcome in most series, and most centers now exclude patients over 50 years old (7,9,10).

2.2 Autologous Bone Marrow Transplantation

Autologous bone marrow transplantation (ABMT) is increasingly used to treat malignant disease (14-32). The underlying therapeutic concept is that storage of marrow harvested in clinical/bone marrow remission allows the patient subsequently to be exposed to treatment that would be lethal were it not for the availability of stored autologous marrow for rescue. The hope is that the increased dose of chemotherapy and radiation therapy will cure a higher proportion of patients than would be possible with conventional therapy. In addition, ABMT may allow the generation of endogenous activated killer cells with antineoplastic function that help eliminate residual leukemia cells (21).

ABMT for acute leukemia is becoming an increasingly common procedure. Preliminary data for ABMT in acute leukemia are encouraging (13,14,17-22,25,28). The 3 year disease-free survival is approximately 30 to 40% for AML patients transplanted in first remission (17,27). The relapse rate is higher for ABMT than AlloBMT, but the treatment related mortality for autologous transplant is less than 10% so that the overall survival for the two types of transplant are similar (11,27).

2.2.1 Use of Busulfan/Cytosan followed by ABMT for AML

In theory, a preparative treatment for autologous transplantation should permit intensification of effective therapies and subsequent rescue of hematopoietic function. The ideal regimen for leukemia should have anti-leukemic effects which include sanctuary areas, yet should permit the autologous marrow to graft and establish normal hematopoiesis rapidly. The combination of busulfan and cytoxan is highly effective as a preparative regimen for AlloBMT in AML and has acceptable defined toxicities (17,24). This combination apparently

eliminates leukemic cells in a high percentage of patients and permits engraftment (24).

A number of studies in AML using autologous marrow engraftment after preparation with busulfan/cytosine have shown promising results (24). In studies from Johns Hopkins Hospital, AML patients in second or subsequent complete remission who had marrow removed, treated with 4HC and then reinfused after treatment with busulfan/cytosine had an estimated event-free survival at 2 years of 30%. By historical review, none of these patients would remain disease free with conventional chemotherapy for relapsed AML more than a median of 6 months (33).

2.3 Mechanism of Relapse

Although ABMT may offer advantages over conventional chemotherapy, the major cause of treatment failure remains relapse or disease progression. When ABMT is undertaken for solid tumors, relapse is generally at the site of original disease, implying that supralethal chemo-radiotherapy has not eradicated the malignancy. The mechanism of relapse following ABMT for marrow cell derived malignancies such as AML is less clear. Two explanations have been offered as to why the incidence of relapse in these diseases is far higher after ABMT than it is after AlloBMT. The first is that the alloreactive T lymphocytes present in allogeneic marrow play a major role in the recognition and elimination of residual host malignant cells (26). Since these cells are absent from autologous marrow, relapse is more likely after ABMT. This explanation suggests that relapse after ABMT for hematological malignancy occurs for the same reason as relapse after ABMT for solid tumor - persistence of residual disease in the host. An alternative explanation is that even though cryopreserved autologous marrow is harvested in remission, it nonetheless contains residual malignant cells. In this case, relapse is due to residual disease not in the host, but in the rescuing marrow (33-35). It is likely that both factors contribute to relapse, although the relative contribution of each in any given disease is unknown.

The mechanism of relapse following ABMT for marrow derived malignancy is an important issue to resolve. The fear that stored marrow contains residual malignant cells has led to intensive investigation of the value of marrow purging prior to storage and subsequent reinfusion. Purging may be undertaken with monoclonal antibodies (35), with cytotoxic agents active *in vitro*, or by physicochemical means (33,34). In animal and pre-clinical human studies all of these methods reduce contamination with malignant cells if these are deliberately added to the marrow, but none have been shown to reduce the risk of relapse in naturally occurring disease. Marrow for ABMT is

harvested in remission when - by definition - no malignant cells are detectable. It had therefore been impossible to undertake any form of quality control to determine if residual malignant cells have genuinely been eliminated. Moreover, there was no evidence that malignant cells with the ability to reestablish the malignancy after ABMT were physically, phenotypically, or biochemically identical to the cells of the "mature" tumor. Until recently, therefore, the justification for bone marrow purging had been largely a matter of faith. This was cause for considerable concern. The techniques of purging almost invariably damage normal progenitor cells, so that the engraftment of purged marrow is generally substantially slower than the engraftment of untreated marrow (33-35). Morbidity and mortality from the complications of hemopoietic and immune system failure were correspondingly increased (10,27).

2.4 Use of Gene Marking to Determine the Need for Purging

Since September 1991, we have been using the LNL6 or G1N retroviruses to mark one third of the marrow harvested for ABMT. These vectors confer Neomycin resistance which can be detected phenotypically or genetically, using the polymerase chain reaction. By November 1992 we had treated 9 AML patient marrows, 5 with LNL6 and 4 with G1N. Two of these patients subsequently relapsed, and in both a proportion of the malignant cells at relapse were marker gene positive. Details of these patients are given in Appendix D. We therefore have unequivocal evidence that marrow harvested in clinical remission of AML contains cells capable of contributing to disease recurrence. Effective marrow purging will therefore be one essential precondition to improving outcome of disease treatment.

2.5 Biology of Autologous Reconstitution

These gene marked marrows also provided information about the mechanisms of marrow repopulation after BMT about which comparatively little had been known. We found that the cryopreserved marrow contained viable long lived "stem cells" which could be marked and which reconstituted the patient. Marked hemopoietic cells - including pluripotent GEMM progenitor cells - have been detected beyond one year post-BMT and T and B lymphoid lineages are also marked at this time (see Appendix E). Thus, the autograft does not simply provide temporary replenishment of committed progenitor cells whilst surviving host stem cells gradually repopulate the individual.

3.0 RATIONALE FOR CURRENT PROPOSAL

The improvements made in autologous transplantation and the outcome for this procedure in patients in second complete remission suggests this may be a good treatment for children with AML. It permits dose escalation and the use of drugs such as busulfan which have excellent antileukemic properties but unpredictable and dose limiting marrow toxicity. Therefore, in this proposal we plan to continue to use ABMT for all children with AML (except those with an HLA-match sibling) in first complete remission.

3.1 Autologous Marrow and Gene Marking

Because the initial studies have shown that residual disease in the harvested marrow contributes to relapse, future autologous marrow transplants will add marrow purging to the treatment regimen. The combination of purging with gene marking allows the efficacy of the marrow cleansing step to be analyzed *in vivo*. Since two distinguishable markers, LNL6 and G1N are available for clinical use, it will be possible to compare the effectiveness of two separate purging techniques *in the same individual*. Two separate aliquots of marrow can be marked with either the G1N or LNL6 vectors and then exposed to one or other of the two purging techniques. The relative proportion of each marker in the blast cells at relapse will be dependent on the relative efficiency of each purging technique.

3.2 Effects of Purging on Autologous Repopulation

As we have shown that the marker gene will successfully be incorporated in long lived pluripotent cells in unpurged marrow, the current protocol will allow us to learn how such cells are affected by each purging technique. We will learn, for example, whether the sometimes profound delay in reconstitution following purging is a consequence of destruction of stem cells, and represents the time taken for residual host cells to recover from "ablative" therapy. We will also be able to compare, in the same individual, the relative effects of each purging technique on the pace and durability of reconstitution.

3.3 Choice of Purging Techniques

Despite many uncontrolled clinical trials, there is still no evidence that any purging technique is clinically effective. There are certainly no data to show that any one technique is superior to any other. To gain the maximum information in this initial study, we have chosen to compare a pharmacologic with an immunologic method of purging. Future studies may use other methods individually, or even in combination.

3.3.1 4-Hydroxy-peroxy-cyclophosphamide (4HC)

This cytotoxic drug is the most widely used agent for purging marrow from patients with AML. Although the drug damages normal progenitor cells and slows engraftment, we have chosen it in part because it represents the closest to a "gold standard" for purging techniques and in part because the institution already has considerable experience with the drug in a Phase 1 study that was part of the AML87 protocol. The drug will be used at a fixed dose in the present investigation.

3.3.2 Interleukin-2 (IL2)

IL2 activated killer cells are able to destroy viable malignant blast cells ex vivo and in vivo in bone marrow. Recently, studies by the Leukemia/Bone Marrow Transplantation program of British Columbia and by the Terry Fox laboratories in Vancouver have shown that ex vivo culture of leukemic patient marrow for 7 days in the presence of IL2 will generate a high level of activated killer cells. IL2 treatment produces little impairment of the subsequent growth of primitive marrow progenitor cells. In a pilot study, 8 of 9 patients receiving marrow cultured ex vivo with IL2 subsequently engrafted. Their median time to 500 neutrophils was 53 days (range 20-71 days). This immunomodulatory approach to marrow purging appears particularly worthy of evaluation, given our previous and current experience with IL2 treatment of patients with AML. Appendix F contains a relevant pre-print.

4.0 STUDY OUTLINE AND SAFETY

This study proposes to transduce marker genes into two separate aliquots of marrow obtained for ABMT in patients in remission of AML. Each aliquot will be purged by a different technique and the marrow cryopreserved. The marrow will be reinfused after the patient has received ablative therapy. The vectors to be used in the transduction studies are the closely related but distinguishable retrovirus vectors LNL6 and G1N. Safety considerations for each of these vectors were discussed in detail in the original AMLREM protocols.

5.0 PLAN OF CLINICAL STUDY

5.1 Specific Inclusion and Exclusion Criteria for Gene Transduction Eligibility (see section 10).

To ensure that sufficient marrow can be purged without potentially compromising the rate of reconstitution should an unpurged backup marrow need to be given, the minimum quantity of marrow mononuclear cells to be obtained at harvest will be $4 \times 10^8/\text{kg}$.

Should this safety margin not be attainable, the patient will be ineligible for the double transduction/purging study and will be treated off protocol as follows:-

If >3 but $<4 \times 10^8$ cells/kg are harvested, the marrow will be purged with 4HC after 10^8 cells/kg are cryopreserved as a backup.

If >1 but $<3 \times 10^8$ cells/kg are obtained the patients will receive unpurged marrow

If $<1 \times 10^8$ cells/kg are obtained the patients will be re-harvested at a later date.

5.2 Preparation of Marrow

Details are provided in technical appendices A and B and shown in diagrammatic form below. In brief, a marrow mononuclear cell fraction will be obtained using a Stericell separator. Two thirds of these cells will be used for cell free vector transduction and purging. The remaining marrow will be processed and frozen as per standard techniques. The marrow for transduction and purging will be divided in two aliquots, one transduced with LNL6 and one with G1N. Each will be transduced at a multiplicity of infection of 10:1 for 6hrs. Each aliquot will then be assigned at random for purging with IL2 or with 4HC. IL2 purging will be the responsibility of the Terry Fox Laboratories, Vancouver. After conditioning, the purged aliquots of marrow will be reinfused. In the absence of engraftment at 40 days (Neutrophils $<100/\text{mm}^3$) patients will receive a 10 course of G-CSF. If ANC remains $\leq 100/\text{mm}^3$, the reserve, unmanipulated marrow aliquot will be returned.

6.0 EVALUATION OF EFFECTS

6.1 Relapse

If relapse occurs, then the malignant blasts will be separated by FACS and it will be determined whether or not the relapse cells contains either marker gene. By using the appropriate primers, the size of the PCR fragment amplified will indicate whether either or both marker genes are present in the bulk population. Semi-quantitative PCR will indicate the relative proportions of each marker gene. Individual clones of leukemic cells will be grown in methylcellulose colonies, and assigned to the LNL6 or G1N aliquot (and hence to one or the other purging methods) on the basis of PCR fragment size.

6.2 Regeneration of Cryopreserved Autologous Marrow

When marrow is obtained to assess engraftment and disease progression, additional cultures will analyze which marker genes are present in progenitor cells and in what lineages. Patients will also be monitored at weekly intervals for 6 weeks, monthly intervals for 6 months and annually thereafter for 5 years for the presence of cells containing the marker genes in the peripheral blood (PB) using FACS sorted populations. PB will be separated into T cells, B cells, monocytes and granulocytes using appropriate monoclonal antibodies (MAb), and each lineage examined for the proviruses. Again, clonogenic analyses of progenitor cells grown in methylcellulose will be used to compare the contribution of each marrow aliquot to reconstitution.

7.0 INFERENCES WHICH MAY BE DRAWN FROM STUDY RESULTS

7.1 Source of Relapse

There are at least three informative results of the study.

- 1) Children relapse with marked cells and both vectors are equally represented.

This result would confirm that purging is necessary but would suggest that the current techniques are inadequate. Studies of new purging methods would be necessary.

- 2) Children relapse and the cells are only marked with the vector associated with one of the two purging methods.

Provided engraftment of normal progenitor cells occurred from both aliquots, this outcome would suggest that one of the techniques is superior to the other and should be explored further in larger scale clinical trials.

- 3) Children relapse and the cells are marked with both vectors, but progenitor representation of each vector is widely disproportionate and there is statistical evidence that one purging technique is more effective than the other.

If no patient relapses with marked cells, then no definitive conclusion could be drawn. However, since the initial studies of this technique have confirmed the feasibility of marking and detecting residual malignant cells in remission marrow this event is unlikely to happen.

8.0 STATISTICAL CONSIDERATIONS

8.1 Potential Accrual and Study Duration

Approximately 25 children newly diagnosed with AML are treated at St. Jude each year. The complete remission rate for AML is presently 75% and thus, we expect 18 children to be available for bone marrow transplantation (BMT). Of these, 30% will have a matched related donor and will be treated with an allogeneic BMT. The remaining 12 children are expected to enroll on this protocol to be treated with an autologous BMT (ABMT).

We propose to treat 35 children on protocol with ABMT in order to observe 14 relapses. Fourteen relapses will provide a reasonable sample size for a first statistical evaluation of the two purging techniques. Furthermore, the sample size of 75 newly diagnosed AML patients treated with BMT will provide at least 80% power for comparison with the historical event-free survival results of AML 87. The sample size of 35 children treated with ABMT will provide at least 75% power for comparing duration of CCR to the historical AML 87 study.

8.2 Safety Monitoring

While there is now considerable experience documenting the toxicity and failure rate of ABMT in this setting as well as the safety of the gene marking techniques, we will, nonetheless, sequentially monitor the study for an unexpected high frequency of these events. A two-staged design will be used with 20 patients being treated in the first stage and 15 patients in the second stage. Since the primary study objective is the evaluation of purging and not

death or toxicity, no adjustment in the primary analysis will be made for the sequential design.

Death during ABMT:

If five deaths during transplantation are observed in the treatment of the first 20 patients, accrual to the study will be terminated and a complete evaluation of all information made as to whether continuation is warranted. This decision rule provides a significance level of 0.17 for the null hypothesis that the true death rate during ABMT is at most 0.15. The power for rejecting the null hypothesis (terminating accrual) if the true death rate is 0.20, 0.30 or 0.40 is 0.37, 0.76 and 0.95, respectively.

Toxicity

If seven episodes of unacceptable (Grade IV) toxicity are observed in the first 20 patients treated, then accrual to the study will be terminated and all data evaluated to determine whether continuation is warranted. This decision rule has a significance level of 0.21 for the null hypothesis that the true toxicity rate is at most .25. The power of rejecting the null hypothesis (stopping accrual) is .75 if the true toxicity rate is 0.40.

If accrual to the study is not halted after 20 patients have been treated with ABMT, the study will continue until a total of 35 patients have been treated with ABMT.

8.3 Evaluation of Purging

If one assume that the study is not terminated early due to safety concerns, then we expect to evaluate the purging techniques in a total of 35 patients. Since the transfection rates may vary with the vectors being used, patients will be randomized to one of the two possible combinations of purging techniques and vectors. A blocking factor of four will be used.

If the transduction efficiency of a vector is assumed conservatively to be 1% and the reinfused marrow is exposed to either the vector G1 or LN6, then the following table provides estimates of the probability of detecting a marked relapse as a function of the number of progenitor cells (NC) giving rise to the relapse. A cell in the exposed marrow has a 0.01 probability of being transfected with one of the vectors. A binomial model has been used to calculate the estimates provided in the table.

NC	Probability of marked relapse
1	0.010
10	0.010
100	0.634
1000	0.999
3000	1.000

Since both relapses observed in our previous study were marked and only 30% of the marrow was exposed to a vector, the above table suggest that it is unlikely that fewer than 100 progenitor cells from the reinfused marrow gave rise to the relapse.

In this study of children with AML, we expect the two year continuous complete remission (CCR) rate to be approximately 60%. Thus we should observe 14 relapses in the 35 patients treated with ABMT. This assumes that ABMT will show no improvement in disease control over the historical data. With the same assumptions used to derive the previous table, the following table provides estimates of detecting at least one marked relapse and the expected number of relapses which will be observed.

NC	Prob (Exp)
1	0.13 (0.1)
10	0.75 (1.3)
100	1.00 (8.9)
1000	1.00 (14.0)
3000	1.00 (14.0)

If we observe 14 unmarked relapses and there is evidence that normal progenitor cells are marked with both vectors, we will be 95% confident that the true failure rate of purging is less than 20%. Note that this result alone does not demonstrate the necessity of purging. That follows from the previous experience of the gene marking study without purging in AML.

Our past experience and the above estimates suggest that there will be 14 relapses in which to evaluate the efficacy of the two purging techniques. For each marked relapse the number of progenitor cells transfected with each vector and giving rise to the relapse will be estimated. The Wilcoxon signed rank test will be used to compare the two techniques in terms of the paired differences between the numbers of progenitor cells marked with each vector. No information exist on which reasonable power calculations can be based. This experiment is considered the first step in investigating these two techniques and based on the observations a definitive trial will be proposed to the Pediatric Oncology Group.

8.4 Historical Control Comparison of Event-Free Survival

The experience of St. Jude Protocol AML-87 will be used as the historical control for evaluating the event-free survival and duration of CCR achieved by ABMT. Kaplan-Meier estimates of duration of CCR for AML-87 are shown in the following table.

YEARS	PROB	SE
2	0.55	0.07
2	0.35	0.06
3	0.32	0.07

Using the technique of Simon and Dixon, we estimate that the study will have approximately 75% power to detect and improvement in duration of CCR from the observes .35 to .55 for ABMT. The test will have a significance level of 0.05 and will be a one sided log-rank comparison.

Kaplan-Meier estimates of both event-free survival and duration of CCR will be calculated.

9.0 DRUG INFORMATION

9.1 Busulfan (Myleran®, BU) NDC# 0081-0713-25

Busulfan is available as Myleran® (Burroughs-Wellcome and Co.) in 2 mg scored tablets. It is impossible to prepare a parenteral form of the drug because it is insoluble in water. Oral preparations are well tolerated. Busulfan for this study will be given four times a day (q 6 hr) at a dose of 4 mg/kg/day PO; four consecutive days prior to CTX administration. The dose is per kg of actual body weight or ideal body weight, whichever is less. Dose

must be taken all at once and may be given through an NG tube. Please consult clinical nutrition (dietitian) for estimates of ideal body weight.

9.1.1 Toxicity (BU)

- a) Hematologic - aplastic anemia.
- b) Addisonian-like syndrome with hyperpigmentation often unassociated with impaired corticosteroid production.
- c) Diffuse interstitial pneumonitis with fibrosis.
- d) Other toxic effects include erythematous skin rash, hepatic dysfunction, amenorrhea, testicular atrophy, gynecomastia, myasthenia symptoms and cataracts.
- e) Nausea and vomiting can occur after busulfan but is generally mild.
- f) Generalized seizures have been reported after use of high dose busulfan. All patients will be treated with dilantin 5 mg/kg/dose (IV or PO) q 6 hr beginning on day -10 for 48 hr (8 doses), then 5 mg/kg daily until day -4. Patients developing seizures should be extensively evaluated and treated as clinically indicated.

9.1.2 Some patients may vomit after administration of busulfan. Since gastric absorption is good and fairly complete within 30 to 45 minutes, the following guidelines should be used to replace doses after emesis.

- a) Emesis within 30 minutes of the busulfan dose repeat one-half of the dose after giving an antiemetic.
- b) Emesis after 30 minutes of the busulfan dose give only the number of tablets found in the emesis.

9.2 Cyclophosphamide (Cytosan, CTX) NSC# 26271

An alkylating agent related to the nitrogen mustards which is biochemically inert but metabolized by liver phosphamidases to active components which are excreted exclusively by the kidney. CTX in the study will be given at a dose of 50 mg/kg IV ideal body weight or actual body weight, whichever is lowest on each of four successive days. CTX is dissolved in 100-250 cc of D5W and

administered over a 30-60 min period followed by IV hydration for 12 hr with 2.4 L/m².

9.2.1 Sedation and antinausea drugs: Ondansetron 0.15 mg/kg IV every 8 hours beginning one hour prior to CTX.

9.2.2 Diuresis and hydration: The urine flow during and for 12 hours after CTX administration is to be kept at a minimum of 2.5 cc/kg/hr. If the urine output decreases below these numbers, furosemide 10-20 mg/m² IV may be given. IV fluids at 2.4 L/m²/12 hr post CTX will be routinely employed. A Foley catheter may be placed in young children if difficulty is measuring urine output occurs.

9.2.3 Toxicity of cytoxan (CTX)

- a. Nausea and vomiting controlled with routine antiemetics.
- b. Fluid retention: CTX has an anti-diuretic effect usually counteracted by furosemide administration. Careful physical examination should be made and daily weights and electrolytes determined to detect fluid overload early.
- c. Cardiomyopathy: At doses greater than 200 mg/kg, CTX can cause fatal cardiac necrosis with heart failure. Patients are monitored by daily EKG's to detect decrease in voltage. Non-specific ST changes are not unusual but a decrease in voltage is significant and ominous. CTX is contra-indicated in patients with existing cardiac disease. The EKG must be checked before each dose of cytoxan, patients developing significant reduction in cardiac voltage will have all subsequent doses discontinued.
- d. Diarrhea: Treated symptomatically.
- e. Hemorrhagic cystitis: Hematuria is not uncommon at this dose level, but is usually not symptomatic or severe. Adequate urine flow is essential to avoid cystitis. All patients will be treated with MESNA coincident with each dose of CTX (see below). Pyridium will given for symptomatic relief.
- f. Alopecia: Usually reversible.

- g. Skin rash: Ten to 20% of patients develop a diffuse maculopapular rash 24-72 hours following CTX. The rash usually clears in 24-48 hours.
- h. Anemia: Hemoglobin drops can occur at this dose, presumably due to hemolysis.
- i. Reproduction: The long term effects are unknown but sterility is probable.

9.3 Mesna (MESNCX) NDC #0015-3561-41

Mesna is a sulfhydryl compound, 2-mercaptoethane sulfonate which has been shown in clinical studies to be effective in preventing CTX induced hemorrhagic cystitis. Mesna combines with and inactivates the metabolites of CTX which are toxic to the mucosa of the bladder. On entering the plasma, Mesna is oxidized to a polar disulfide (dimesna) which does not react with cytoxan metabolites. The kidney then transforms dimesna back to mesna which inactivates cytoxan metabolites in the urine. Thus, Mesna does not interfere with the antineoplastic and immunosuppressive effects of cytoxan but does selectively protect the bladder epithelium from its toxic metabolites.

9.3.1 Dosage:

- a. Supplied as 400 mg/4 ml vial.
- b. Dissolved in D₅W to 20 mg/ml concentration.
- c. Mesna 12 mg/kg is delivered IV over 15 minutes prior to CTX and 3, 6, 9, and 12 hours after each dose of cytoxan.
- d. Total 20 doses Mesna (5 for each dose of CTX) will be administered with BU/CTX preparatory regimen.

9.3.2 Toxicities:

In general, adverse reactions to mesna have been mild and readily managed including headaches, arthralgia and diarrhea. Hypotension and allergy were reported in 1/6 patients on phase I study.

9.4 4-Hydroxyperoxy-cyclophosphamide

Pergamid™ (4-Hydroperoxycyclophosphamide) is supplied by Nova Pharmaceutical Corporation as a sterile powder in vials containing 200 mg 4-

hydroperoxycyclophosphamide for ex vivo use. Pergamid™ is soluble in water, saline, or tissue culture medium.

9.4.1 Indication

Pergamid™ is indicated for the ex vivo pharmacologic purging of autologous bone marrow of residual neoplastic cells.

9.4.2 Dosage and Use

Bone Marrow Purging: The autologous bone marrow sample should be treated with Pergamid™ at a final concentration of 60 µg/ml.

9.4.3 Preparation and Handling of Solutions

Pergamid™ should be stored at or below -20°C in a desiccator. The desiccator should be at room temperature for one-half hour prior to opening.

Pergamid™ should be reconstituted by adding 20 ml sterile saline or tissue culture medium to the 50 ml vial and shaking until all particulate material has disappeared. This results in a concentration of 10 mg Pergamid™ per ml of solution in the vial. For details of marrow treatment see Appendix A.

9.4.4 How Supplied

Pergamid™ is supplied in sterile vials for single use ex vivo containing 200 mg 4-hydroperoxycyclophosphamide. Storage at or below -20°C is required. Pergamid™ solutions should be used within one hour of reconstitution.

9.5 Interleukin-2

9.5.1 Storage stability of nIL2 Biotest

nIL2 Biotest can be stored for over a year at temperatures in the range from +2°C to +8°C. From preliminary storage tests carried out at other temperatures, it can be assumed that the nIL2 preparation can be stored for up to 6 months at temperatures in the range from +15°C to +25°C (room temperature). Storage at higher temperatures is inappropriate.

9.5.2 Biotest nIL2 is provided in liquid form in ampoules of 1 ml (10⁵ units).

- 9.5.3 The preparation should be stored in a refrigerator at 2°C. However, short-term storage at up to 37°C has no detrimental effect on the activity of the IL2 or units storage stability. The preparation should be warmed to room temperature prior to use.
- 9.5.4 Repeat freezing and thawing out reduces the activity of the IL2.
- 9.5.5 Broken ampoules should be used immediately.
- 9.5.6 nIL2 is inactivated at pH values above 10 and below 2.
- 9.5.7 All surface contact with glass or plastic results in nIL2 losses. By way of example, a tuberculin syringe absorbs about 10% of the given activity of the nIL2.
- 9.5.8 Sterile filters absorb nIL2. Slight losses occur in the even of filters with low protein absorption being used, e.g. Durapore filters from Millipore or Gelman sterile filters. The filters should be coated with a protein solution - e.g. serum or 0.1% HSA - prior to use.
- 9.5.9 Dilutions below 10^5 U/ml cause considerable IL2 loss. This can be avoided by introducing into the filuent (phosphate butter solution) as a stabilizing agent 0.1% human serum albumin.
- Absolutely essential: introduce the human serum albumin into the phosphate buffer solution first, shake, and only then add the nIL2.
- 9.5.10 Avoid oxidation losses.

10.0 PATIENT ELIGIBILITY

- 10.1 Patients aged between 1 and 18 years at diagnosis with acute nonlymphocytic leukemia in first remission are eligible for this protocol.
- 10.2 Patient exclusion includes patients with:
1. HLA-matched, MLC-compatible donor (unless parents and/or patient refuses allogeneic transplant)
 2. life expectancy limited by disease other than leukemia
 3. significant cardiac disease (echo shortening fraction <25% or MUGA scan <50%)

4. severe renal dysfunction, i.e., creatinine clearance more than twice normal for age
5. severe restrictive pulmonary disease (FVC less than 40% of predicted)
6. severe hepatic disease (bilirubin greater than 3 mg/dl or SGPT greater than 500 IU)
7. severe personality disorder or mental illness
8. previous severe cystitis from cyclophosphamide
9. previous total dose of anthracyclines of $>450 \text{ mg/m}^2$
10. severe infection that on evaluation by the Principal Investigator precludes ablative chemotherapy or successful transplantation
11. Previous allogeneic or autologous transplant
12. HIV reactivity
13. Karnofsky score $<70\%$.

11.0 OUTLINE OF THERAPY

11.1 Harvest guidelines:

Patients will enter the study at the time of bone marrow harvest. Bone marrow must be obtained and frozen during a complete remission. Transduction will be done on 66% of the harvested marrow before the cells are frozen and stored. At least 1×10^8 cells/kg must be available as untreated back-up and at least 3×10^8 nucleated cells/kg must be available for the marking purge. It is anticipated that patients will have their bone marrow harvested and then proceed directly to conditioning and transplantation. Criteria for marrow harvest include:

- 11.1.1 Pre-harvest bone marrow aspirate and biopsy performed within 3 weeks of harvest must demonstrate adequate cellularity (based on bone marrow biopsy cellularity of greater than 40%, taking into account sampling error), and no evidence of leukemia ($<5\%$ blasts and absence of leukemic markers, such as Auer rods).

11.1.2 Cytogenetic analysis performed on the pre-harvest bone marrow must be normal.

11.1.3 Cell harvest must be sufficient for marrow reconstitution (cell yield must be greater than 4×10^8 nucleated cells/kg). This should yield sufficient cells for bone marrow transplantation even if the cells taken for vector marking/purging cannot be used.

11.2 Patients without CNS leukemia will receive busulfan (1 mg/kg of ideal or actual body weight whichever is less) orally every 6 hours for 16 doses on days -9, -8, -7, -6, followed by cyclophosphamide (50 mg/kg/dose) with Mesna on days -5, -4, -3, -2. Patients will also receive dilantin to prevent busulfan-induced seizures. Autologous marrow is infused 48 hours later.

<u>Protocol day</u>	<u>Treatment</u>
10	Begin Dilantin (5 mg/kg q 6 h x 8 doses) then 5 mg/kg/day to day -4
-9	BU
-8	BU 1 mg/kg, q 6 hr x 16 doses
-7	BU
-6	BU
-5	CTX 50 mg/kg + MESNA
-4	CTX 50 mg/kg + MESNA (stop Dilantin)
-3	CTX 50 mg/kg + MESNA
-2	CTX 50 mg/kg + MESNA
-1	REST
0	Autologous bone marrow reinfusion

11.3 Patients with CNS disease documented by lumbar puncture on pretransplant evaluation, will receive IT methotrexate (dose below) every third day until clear x 2 (minimum number = 3, maximum number = 5). Three days after the last lumbar puncture patients will begin BU treatment as outlined above. If the IT methotrexate results in headache, photophobia, or neck stiffness then 20 mg of hydrocortisone will be added.

<u>Age (yr)</u>	<u>MTX (mg)</u>
<1	6
1-2	8
2-3	10
>3	12

Patients with a history of CNS disease at any time in the past (including immediately pre-transplant) will also receive 5 lumbar punctures with IT

methotrexate (as above) post transplantation when WBC $>2.0 \times 10^9/L$ and platelets $>75 \times 10^9/L$. These 5 LPs will be performed over 2½ to 4 weeks.

Citrovorum will be given 5 mg PO (or IV) every 6 hours x 4 starting 24 hours post IT MTX. If arachnoiditis develops, all IT MTX will be given with 20 mg of IT hydrocortisone.

- 11.4 BU (busulfan) = 4 mg/kg/day (total 16 mg/kg). Daily dose divided into every 6 hour oral doses (1 mg/kg every 6 hours). Pills (2 mg) may be enclosed up to 8 per gelatin capsule (16 mg).

DILANTIN: Since generalized seizures have been reported with high dose busulfan, patients will receive prophylactic dilantin 5 mg/kg/dose (IV or PO) q 6 hr starting day -10 for 48 hr (8 doses) then 5 mg/kg daily until day -4.

CTX (cyclophosphamide) = 50 mg/kg IV on each of four successive days.

Hematuria is not uncommon at this dose level of cytoxan. Adequate urine flow is essential and the exact hydration and MESNA doses should be followed. Please see agent information section VI and preprinted order sheets.

11.5 Marrow Infusion

At the time of autologous marrow reinfusion (day 0), the LNL-6 and G1N purged bone marrow suspension will be rapidly thawed in a 37° water bath and each bag rapidly infused through a central venous catheter.

- 11.5.1 Marrow to be infused will arrive on the unit hand-carried by the transplant coordinator.

NOTE: Marrow DOES NOT receive IRRADIATION.

11.5.2 Preparation of the patient

- a. Oxygen and suction equipment MUST be set up in room.
- b. Emergency drugs BENADRYL, EPINEPHRINE, SOLUMEDROL OR SOLUCORTEF in appropriate doses must be ordered by the physician prior to initiation of infusion and be available at the bedside. See preprinted order sheets for marrow reinfusion.
- c. Procedure is explained to the patient and family.

- d. All patients will have central venous access, preferably a double lumen catheter system.
- e. VS flow sheet at the bedside
- f. Normal saline for IV infusion
- g. Baseline vital signs are taken and recorded.
- h. Patients receive a pre-med with Benadryl and hydrocortisone 15 minutes prior to infusion.

11.5.3 Marrow Infusion

- a. After thawing rapidly in 37°C water bath, the marrow is administered by the physician as rapidly as possible. A physician and nurse both MUST remain in the room for the total infusion time and one or the other for 1 hr post infusion.

NOTE: The marrow MUST NOT be FILTERED.

- b. Vital signs are taken q 15 min. x 4; Q 30 min. x 4, Q hour x 4 and recorded. Patient is monitored by oximeter during infusion and for 6 hours afterward.

11.5.4 Toxicity of Infusion

- a. Volume overload:

This is of particular importance in small recipients.

- b. Pulmonary emboli

Rare complication that might occur due to fat and marrow cell emboli. Marrow and fat emboli cause a transient alveolar capillary block and administration of O₂ or IPPB may be necessary.

- c. Allergic reactions

Chills, fever and hives occasionally occur. These reactions are never severe and respond to additional parenteral diphenhydramine (benadryl).

- d. Renal failure from hemoglobinuria. Urine output must be carefully monitored.

12.0 CONCURRENT TREATMENT AND SUPPORTIVE CARE

- a. Irradiate all blood products (except the bone marrow) with 1500-3500 cGy.
- b. Leukocyte poor RBCs and platelet components will be used to minimize transmission of CMV.
- c. Allergic reactions (especially to platelet concentrates) can usually be controlled with oral or IV benadryl.
- d. Platelets: Irradiated leukocyte poor platelet concentrates are transfused to prevent bleeding and in an attempt to keep the circulating platelet count at or greater than $20,000/\text{mm}^3$ for the first 3-4 weeks post-BMT.
- e. Red cells: PRBCs (irradiated, leukocyte poor) 10-15 cc/kg/dose will be administered to keep the hemoglobin level ≥ 10 g/dl. Higher hemoglobin concentrations might be considered for patients with pulmonary insufficiency.

12.1 Hyperalimentation

Adequate alimentation appears to be an essential for successful transplantation. Parenteral hyperalimentation is maintained until normal enteral alimentation can be resumed as judged by the clinical situation. Patients may be enrolled on the active SJCRH hyperalimentation protocols and will be monitored by the SJCRH Metabolic Support Service.

12.2 Management of infections

- a. Cultures are obtained as clinically indicated.
- b. Once antibiotic treatment has been initiated, it will not be routinely discontinued until marrow function has been restored, even if clinical evidence of infection, i.e., fever, has resolved.
- c. Special emphasis will be placed on evaluation of infection at sanctuary sites such as the sinuses.
- d. Antifungal treatment will be considered in febrile patients who have had 4 days of broad spectrum IV antimicrobials. Patients may be enrolled on open SJCRH infectious disease protocol for treatment.

- e. High index of suspicion is necessary for evaluation and treatment of interstitial pneumonitis in the post-BMT patient. Patients with low-grade fever and chronic cough should have prompt chest x-ray and pulmonary evaluation. FVC will be obtained two times a week for the first three weeks post-BMT and oximeter studies will be employed as necessary.
- f. To prevent activation of pneumocystis carinii (PCP) all patients will receive oral prophylaxis with trimethoprim (150 mg/m²/day) plus sulfamethoxazole (750 mg/m²/day) in two equally divided doses on Monday, Tuesday and Wednesday until day -1. Oral prophylaxis with trimethoprim/sulfamethoxazole will resume when the ANC >500/cu mm.

13.0 EVALUATION DURING THERAPY

13.1 Pre-Transplant

1. History - complete history of previous antineoplastics, radiation therapy, infections, and transfusions
2. Complete physical examination
3. Laboratory screen
 - a. CBC, differential, platelet estimate
 - b. Electrolytes, BUN, creatinine, SGOT, SGPT, gamma GT, total bilirubin, total protein, albumin, alkaline phosphatase
 - c. Urinalysis
 - d. Chest x-ray
 - e. Echocardiogram and EKG
 - f. CPK isoenzymes, LDH
 - g. Baseline pulmonary function tests (arterial blood gases if indicated)
 - h. Coagulation screen

- i. Cytotoxic antibody screen
 - j. Surveillance cultures
 - k. HIV, CMV, hepatitis, A, B and C screens
- 4. Bone marrow aspirate and biopsy
 - 5. Lumbar puncture for culture and cytology
 - 6. Bone marrow for karyotype analysis
 - 7. Skin test: multi-test unit (by IDS)
 - 8. 5 cc reference serum
 - 9. If available, bone marrow cells and peripheral blood leukocytes will be stored for future studies.

13.2 During and Post-Transplantation (Months 0-3)

- 1. A daily CBC, differential and platelet estimate until self-sustaining and then as indicated.
- 2. Daily electrolytes during cytoxan and at least weekly SGOT, SGPT, total bilirubin, alkaline phosphatase for the first 45 days.
- 3. EKG on days -5, -4, -3, -2, before each dose of cytoxan, and again on days 1 and 7 post-marrow reinfusion.
- 4. Viral, bacterial and fungal cultures as clinically indicated.
- 5. Chest x-ray weekly for 3 weeks and as indicated.
- 6. Bone marrow aspirate and biopsy days +21, +32 and day +100 post marrow reinfusion for morphology and as indicated, plus neo-gene content on colony culture. Lumbar puncture at the end of the third month.
- 7. Patients with CNS involvement will receive 5 spinal taps with methotrexate (see Section IV.C.2.) after hematopoietic recovery.

13.3 Evaluation after Hematologic Reconstitution.

1. Weekly for 6 weeks, monthly for 6 months and every 6 months for 2 years, then annually for 2 years, analysis and separation by FACS of peripheral blood nucleated cells with sorting into granulocytes, monocytes, T cells and B cells and subsequent analysis for marker gene insertion (see protocol). Such studies should limit the amount of blood removed to 20-40 ml. Serum will also be frozen and sent for analysis for replication competent virus (all samples to BMT lab).
2. Patients will also undergo complete physical examination, complete blood counts, and bone marrow aspiration every 6 months for the first 2 years and annually thereafter for the next 2 years. Serum will be sent for analysis of replication competent virus.
3. CSF⁺ patients will require a lumbar puncture every 6 months for the first 2 years, then annually for 2 years.
4. Patients will be evaluated annually for the next 10 years by complete physical examination and complete blood counts. Serum will be sent for analysis of replication competent virus.
5. Patients will be asked to return thereafter at any time if they develop any hematologic/immunologic abnormality or if any malignancy develop. Malignant tissues will be examined for proviral (monoclonal) insertion.

14.0 RESPONSE CRITERIA

All patients will be transplanted in complete remission. Response criteria will be measured by event-free and disease-free survival.

15.0 OFF STUDY CRITERIA

1. Relapse of AML that required retreatment
2. Death from transplant related causes
3. Inability to clear CSF blasts with IT MTX prior to ABMT.

16.0 RECORDS TO BE KEPT

All required clinical evaluation records will be the responsibility of Drs. Malcolm Brenner, Robert Krance and Helen Heslop. Analysis of the clinical outcome and toxicity will also be their responsibility. (see Section 13.0).

The laboratory evaluation of retroviral insertion will be the responsibility of Drs. Brenner and Ihle.

Toxicity will be evaluated by the NCI Common Toxicity Criteria (Appendix D) and the Dartmouth Hematologic Transplant Criteria (Appendix E).

Adverse Reaction Reporting Requirements

This protocol uses retroviral-mediated genetic material which is considered an IND agent. Toxicities occurring on this treatment should be considered IND related.

The following adverse reactions must be reported to the NIH Office of Protection from Research Risks (OPRR) and the local IRB in the following manner described. In addition, a copy of the report will be forwarded to the NIH Office of Recombinant DNA Activities and the US Food and Drug Administration.

Deaths

Any death from any cause while a patient is receiving treatment on this protocol or up to 30 days after the last dose of protocol treatment, or any death which occurs more than 30 days after protocol treatment but which is felt to be treatment related, must be reported as follows:

A telephone call must be made to the OPRR, Charles R. McCarthy, (301) 496-7005, within 24 hours of the event. An Adverse Drug Reaction Form must be sent to the NIH Office for Protection from Research Risks within 10 working days.

Charles R. McCarthy
OPRR
Building 31, Room 5B-59
NIH
Bethesda, MD 20892

Life-Threatening Events (Grade 4) [ECOG Common Toxicity Criteria (Table 4)].

A telephone call must be made to the OPRR, Charles R. McCarthy, (301) 496-7005, within 24 hours of any grade 4 toxic event which may be due to the IND agent. An Adverse Drug Reaction Form must be sent to the OPRR within 10 working days.

First Occurrence of a Toxicity

The first occurrence of a toxicity (not previously reported, no matter what the grade (1-5), must be reported by phone to the OPRR, Charles R. McCarthy (301) 496-7005, within 24 hours of the event. An Adverse Drug Reaction Form must be sent to the OPRR, Charles R. McCarthy, within 10 working days.

17.0 INFORMED CONSENT

All patients entering this study must give written, informed consent. In addition, patients between the ages of 7 and 18 should sign the Assent form appended. Because of the investigational nature of the gene marking procedure, all informed consent should be obtained in the presence of both a study investigator and an individual not directly involved with the protocol. In the event of disparity between the wishes of the child and the wishes of the legal guardian, a member of the Social Work or Psychology Departments will act as patient advocate and witness the consent/assent.

18.0 SUMMARY STATEMENT; PART 1

BONE MARROW TRANSPLANTATION

You are (your child is) in remission from acute myeloid leukemia (AML). But there is about a 65% chance that the leukemia will return. Once AML comes back it is very hard to treat. We believe that the best way to prevent this is to give you (your child) very high doses of chemotherapy to kill any leukemia cells that may be left. Then, a bone marrow transplant will be done using your (your child's) own bone marrow. This is necessary because the chemotherapy also kills normal cells in the marrow.

STUDIES BEFORE TREATMENT

To make sure the transplant is safe, we will test your (your child's) blood for certain viruses. These include hepatitis B, which causes liver damage; cytomegalovirus, which causes lung damage; and HIV, which causes AIDS. If these tests show that any of the viruses are there we will discuss with you how they might affect your treatment. We will also do other tests to help us plan your transplant.

MARROW HARVEST

Before the high-dose chemotherapy starts, we will remove a small part of your (your child's) bone marrow from the hip bone. This will be done in the operating room under general anesthesia. You (your child) will not feel anything when the marrow is taken. There may be some pain later. You (your child) will be given medicine for the pain.

HIGH-DOSE CHEMOTHERAPY

Soon after bone marrow harvest, chemotherapy will start. A drug called busulfan will be given by mouth four times a day for four days. The drug Cytosan will be started on the day after the last dose of Busulfan. It will be given by vein (IV) for four days. If there are leukemia cells in the spinal fluid before treatment, the drug methotrexate will be given in the spinal fluid every 3 days until the cells are killed. The maximum number of spinal taps will be 5.

BONE MARROW TRANSPLANT

You (your child) will rest for one day after the chemotherapy. Then the bone marrow that was taken out before will be put back through an IV catheter. Bone marrow aspirates and biopsies will be done on days 21 and 32 after the transplant. Bone marrow aspirates will also be done after you are (your child is) well to see how the graft is working. We will take these samples every 6 months for 2 years and then once a year for 2 years. Any marrow that you do not need (your child does not need) may be used for research.

CONFIDENTIALITY

Your (your child's) case will be treated in complete confidence, unless you specifically agree otherwise. However, approved people from Government agencies may review the charts.

SIDE EFFECTS

The drugs in this study will cause some bad side effects. Doctors will watch you (your child) carefully to prevent these effects or make them less severe. You (your child) will receive a drug called Mesna to prevent bleeding from the bladder, and Dilantin to stop possible seizures (fits). Bone marrow transfusion can also produce very serious side effects. The busulfan and Cytosan destroy normal bone marrow and can cause severe infections and bleeding. You will need antibiotics, blood transfusions and possibly a medicine called

amphotericin B for fungal infections. Also, the transfused marrow does not "take" in some cases (1 in 10 cases at other centers). If this happens, a second marrow transfusion may be given. The table below lists the possible short-term and long-term side effects.

AGENT	POSSIBLE SIDE EFFECTS
Busulfan	Permanent damage to bone marrow Increased skin pigmentation (color) Lung disease Skin rash Liver damage Inability to have children (sterility) Breast growth in males Cataracts Second cancers Seizures
Cytosan	Severe nausea and vomiting Electrolyte imbalance and fluid retention Diarrhea Bleeding from the bladder Hair loss Skin rashes Low blood counts Sterility Heart failure
Methotrexate (injected in the spinal fluid)	Headaches Nausea, vomiting Seizures ("fits") Brain damage Back pain
Dilantin	Sleepiness Gum problems Allergies
Mesna	Headache Joint pain Diarrhea

ALTERNATIVE PROCEDURES

If you do not want a transplant for yourself (your child), we could give treatment with different drug combinations or radiation therapy. Although the side effects would not be as bad, we do not think these treatments would control the cancer better. Also, you (your child) could have no further therapy.

STATEMENT OF UNDERSTANDING

The benefits of this experimental treatment are difficult to predict. From experience at other childhood cancer centers, we expect that 1 of every 2 children treated this way will remain free of leukemia for at least 2 years after the completion of high-dose chemotherapy and bone marrow transfusion.

Participation in the study is strictly voluntary. If you/your child does not wish to volunteer, other options will be discussed and made available. Should any problems arise, one of the doctors will make you/your child aware of these developments and discuss plans of action.

1. I have read the above treatment and voluntarily agree to the participation of myself/my child _____ in this study/treatment.
2. I have had adequate opportunity to discuss with Dr. _____
name of investigator
caring for me/my child _____ all the purposes and hazards related to this study/treatment.
3. I have been told of alternative courses of action in my/my child's situation.
4. I understand that the study/treatment proposed may result in risks that are currently unforeseeable.
5. I understand that I may withdraw myself/my child from this study/treatment at any time without jeopardy to further treatment.
6. I understand that without marrow transfusion, chances for surviving chemotherapy with busulfan and cytoxan are very poor.
7. I understand that financial compensation is not provided for participation in this study/treatment.
8. I understand that in the event of non-negligent physical injury to myself/my child from this study/treatment, financial compensation is not available but medical treatment necessary will be provided at no direct cost.
9. I understand that information obtained will be kept confidential but that supervised review by approved medical representatives of outside institutions or agencies may occur. In particular, the chart may be reviewed by an agent of the Food and Drug Administration, the National Cancer Institute or their agent, and the Clinical Trials Monitoring Service. No information will be released to the press without my/my child's permission.

10. I understand that if I have further questions regarding this study/treatment or concern about injury from this study/treatment, I can contact Drs. Robert Krance, Malcolm Brenner or Helen Heslop at (901) 522-0300.
11. I understand that further information regarding my/my child's rights as a research participant can be obtained by contacting the Chairman of the Clinical Trials Review Committee, at (901) 522-0300.
12. I understand that I can receive a signed copy of this summary statement and a separate detailed treatment plan, if I wish.

Patient

Parent or Guardian

Investigator

Witness

Date

Time

19.0 SUMMARY STATEMENT; PART 2

GENE MARKING AND MARROW PURGING

You are (your child is) in remission from acute myeloid leukemia (AML). But there is about a 65% chance that the leukemia will return. Once AML comes back it is very hard to treat. We believe that the best way to prevent this is to give you (your child) very high doses of chemotherapy to kill any leukemia cells that may be left. Then, a bone marrow transplant will be done using your (your child's) own bone marrow. This is necessary because the chemotherapy also kills normal cells in the marrow.

Although you have agreed to this treatment, the leukemia may still come back, in some cases because the bone marrow we put back contains hidden leukemia cells. We would like you (your child) to be in a research study to find out if drug treatment of the marrow ("purging") before we put it back can remove these leukemia cells. To do this, we will split the marrow into three parts and put a different genetic marker into two of them. One marked part will then be treated with the drug 4HC, the second with the drug Interleukin-2, and the last part will be frozen without treatment. Both parts of the marrow will then be returned to you (your child). Each step of the treatment plan is given below.

MARROW HARVEST AND MARKING

Before the high-dose chemotherapy starts, we will remove a small part of your (your child's) bone marrow from the hip bone. This will be done in the operating room under general anesthesia. You (your child) will not feel anything when the marrow is taken. There may be some pain later. You (your child) will be given medicine for the pain.

If you agree (your child agrees) to be in the "purging" study, we will freeze about one-third of the marrow immediately with no treatment. If necessary, this will be used as a back-up. Half the remaining marrow will be marked with LNL6 and half with GIN. These are the names of special mouse viruses that have been changed to keep them from causing infection. The marker, a bacterial gene called "neoR", is put inside these special viruses. One half of the marrow will then be treated with a drug called 4-HC and the other with a drug called Interleukin-2. We think, but we do not know, that both of these treatments will help remove leukemic cells from your marrow. The markers let us follow the treated marrow in your (your child's) body to see if these drugs really do remove leukemia cells from the marrow, and to see which of them works best.

RISKS

Purging

The major risk of "purging" is that the normal cells in marrow are also damaged by the drugs so that the marrow is very slow to recover. This increases the risk of infection and bleeding. Sometimes the marrow does not recover at all. In that case we will give back the portion of your/your child's marrow that was frozen without treatment.

Marking

No bad effects have been seen in animal studies using these markers or in any person treated with marker genes here or at other institutions. Still, there may be risks. It is

possible that the mouse virus might "recover" in the cell and be able to grow; it might even cause cancer. We think this is very unlikely but we cannot rule it out yet.

BENEFITS

The benefits of this purging/marker study would be that we could learn what methods of purging work best. This will help us plan better ways of treating AML. If you (your child) needed another transplant in the future, these changes might help you (your child). Otherwise, what we learn might lead to better treatment for other patients with AML.

BONE MARROW TRANSPLANT

You (your child) will rest for one day after the chemotherapy. Then the bone marrow that was taken out before will be put back through an IV catheter. So that we can study the marked cells, we will take a little extra blood after the marrow is put back. The extra blood taken will be about 20 mL (1 tablespoon) once or twice a week for 6 weeks. We will also take extra blood monthly for 6 months, every six months for 2 years, and then once a year for 2 years.

LONG-TERM FOLLOW UP

We will follow your (your child's) progress over a long period. You (your child) will need to be examined yearly for 14 years after the transplant. After the first 4 years this can be done by your (your child's) local doctor. A teaspoon of blood will be taken at each exam.

CONFIDENTIALITY

Your (your child's) case will be treated in complete confidence, unless you specifically agree otherwise. However, approved people from the Food and Drug Administration and National Institutes of Health, as well as St. Jude clinical staff may review the charts.

SIDE EFFECTS

Purging

May delay or prevent the graft from taking. May increase the risk of infection or bleeding.

Virus - No known side effects.

(Possibly could cause a few antibiotics such as neomycin or amikacin not to work or the virus could "recover" and grow or could make cells cancerous)

ALTERNATIVES TO PURGING/MARKING

If you do not wish to have purging/marking treatment you/your child can still have high-dose chemotherapy and bone marrow transplant with untreated marrow.

If you wish to have purging but not gene marking your/your child's marrow can be treated with the drug 4HC alone.

19.1 STATEMENT OF UNDERSTANDING

The benefits of this experimental study are difficult to predict, and it is possible that no useful information will be gained. Participation in the study is strictly voluntary. If you/your child does not wish to volunteer, other options will be discussed and made available. Should any problems arise, one of the doctors will make you/your child aware of these developments and discuss plans of action.

1. I have read the above treatment and voluntarily agree to the participation of myself/my child _____ in this study/treatment.
2. I have had adequate opportunity to discuss with Dr. _____
name of investigator
caring for me/my child _____ all the purposes and hazards related to this study/treatment.
3. I have been told of alternative courses of action in my/my child's situation.
4. I understand that the study/treatment proposed may result in risks that are currently unforeseeable.
5. I understand that I may withdraw myself/my child from this study/treatment at any time without jeopardy to further treatment.
6. I understand that financial compensation is not provided for participation in this study/treatment.
7. I understand that in the event of non-negligent physical injury to myself/my child from this study/treatment, financial compensation is not available but medical treatment necessary will be provided at no direct cost.
8. I understand that information obtained will be kept confidential but that supervised review by approved medical representatives of outside institutions or agencies may occur. In particular, the chart may be reviewed by an agent of the Food and Drug Administration, the National Cancer Institute or their agent, and the Clinical Trials Monitoring Service. No information will be released to the press without my/my child's permission.
9. I understand that useful information may or may not be generated from my participation in this study.
10. I understand that if I have further questions regarding this study/treatment or concern about injury from this study/treatment, I can contact Drs. Robert Krance, Malcolm Brenner or Helen Heslop at (901) 522-0300.

11. I understand that further information regarding my/my child's rights as a research participant can be obtained by contacting the Chairman of the Clinical Trials Review Committee, at (901) 522-0300.
12. I understand that I can receive a signed copy of this summary statement and a separate detailed treatment plan, if I wish.

Patient

Parent or Guardian

Investigator

Witness

Date

Time

ASSENT FORM - PATIENT AGE 7-18 YEARS FOR BONE MARROW TRANSPLANT

ASSENT

You are in remission from AML. This means we cannot see any leukemia cells in your bone marrow or spinal fluid. There is a good chance your leukemia will return if you do not have further treatment. We would like to stop your leukemia from coming back by performing a bone marrow transplant using your own bone marrow. Before we do the bone marrow transplant we will examine you to be sure you can have the transplant. We will also test you for certain viruses including the virus for AIDS.

We will take a small part of your bone marrow out while you are asleep in the operating room. Since you will be sleeping, you will not feel us take the marrow. When you wake up, you may have some pain but you will receive medicine to make this feel better. Your bone marrow will be frozen for later use.

At a later time, you will come into the hospital and receive high-dose anticancer drugs that will stop your blood-forming cells from growing. We hope these drugs will get rid of your leukemia and stop it from coming back. We will then thaw your marrow and give it back to you so your blood forming cells will grow again.

MARROW PURGING AND MARKING

If you agree, we will take a part (two-thirds) of the bone marrow that we have removed and mix it with special viruses. These special viruses are like a taxicab and drop a marker in your cells. We can watch the marked cells grow back after your bone marrow transplant. We will then treat your marrow with two drugs to try and kill any leukemia cells left behind in your marrow. We hope that the markers will let us learn more about your leukemia and our treatment and may one day help us to better treat you or others.

HIGH-DOSE CHEMOTHERAPY

You will receive a drug called busulfan by mouth 4 times each day for four days (total 16 times). You will then receive a second drug, called Cytosan, by vein daily for four days. If leukemic cells are found in your spinal fluid, you will also have spinal taps with a drug called methotrexate.

BONE MARROW TRANSPLANT

After the high-dose drug therapy you will have a day of rest. Then your frozen bone marrow will be thawed and given back to you by your central catheter. An extra amount of blood will be taken once or twice a week for six weeks, and then monthly for six months, every six months for two years, and once a year for two more years. This will help us to test how the marrow cells are growing. You will also have a total of eight bone marrow aspirates to see how well your marrow is growing and whether the marked cells are present. If you had leukemic cells in your spinal fluid before transplant, you will have an additional five spinal taps after the transplant is completed. The maximum number of spinal taps you will have is 10 (unless your leukemia returns).

LONG TERM FOLLOW-UP

We will follow your progress over a long period. You will be examined here each year for four years after the treatment. For the next 10 years, your yearly exams can be done by your local doctor. At each exam, a teaspoon of blood will be taken.

SIDE EFFECTS

The drug treatment and transplant can produce bad side effects. These include permanent damage to the bone marrow, lung disease, liver disease, other cancers, severe vomiting, diarrhea, bleeding from the bladder, hair loss, serious infections, fevers, headaches, seizures. You may not be able to have children after this treatment. The special virus has not produced any bad side effects in anybody. It is possible that it might recover and grow, or stop some antibiotics from working or even cause cancer. We do not think this will happen, but it is possible.

ALTERNATIVE PROCEDURES

If you do not want to have your bone marrow marked you may have a bone marrow transplant without the marking. If you do not want your marrow "purged", you may have a bone marrow transplant without purging. You can also receive other drugs and radiation therapy or you could have no treatment at all. We believe that a transplant is most likely to cure you.

STATEMENT OF UNDERSTANDING

All information about your treatment will be kept among the hospital staff that need to know. We will not tell anybody else without asking you first. You have the right to ask any questions you want and they will be answered. You have had this assent form read or discussed with you by your parents and the doctor (Dr. _____) caring for you. Before you signed this form you had all your questions answered.

If you have any other questions you can ask your doctor or Drs. Robert Krance, Malcolm Brenner or Helen Heslop at any time.

___ I agree to have my marrow marked.

___ I do not agree to have my marrow marked.

Patient

Parent or Guardian

Physician

Witness

Date

Time

REFERENCES

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BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME	POSITION TITLE		
BRENNER, Malcolm Keith	Member, Hematology/Oncology		
EDUCATION (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)			
INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
University of Cambridge	MA, MB, BChir	1975	Medicine
Membership, Royal College of Physicians	MRCP	1977	Medicine
University of Cambridge	PhD	1981	Immunology
Membership, Royal College of Pathologists	MRCPath	1988	Haematology
Fellowship, Royal College of Physicians	FRCP	1990	Medicine

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

1975 House Physician to Professor M.D. Milne, Westminster Hospital, London, U.K.
 1976 Senior House Officer to Professor J.P. Shillingford, Hammersmith Hospital, and Royal Postgraduate Medical School, London
 1977 Senior House Officer to Professor M. Turner Warwick and Dr. P. Cole, Brompton Hospital, London
 1977-78 Medical Registrar to Professor A.F. Lant and Dr. L.W. Loughridge, Westminster Hospital, London, U.K.
 1978-81 Medical Research Council Training (MRC) Fellow, Division of Immunology, Department of Pathology, Cambridge University, U.K.
 1981-83 MRC Clinical Scientist, Honorary Senior Registrar, Northwick Park Hospital, and Clinical Research Centre, London
 1983-85 Lecturer in Haematology, Royal Free Hospital and Hospital for Sick Children, Great Ormond Street, London
 1985-89 Senior Lecturer, Honorary Consultant Haematologist (Deputy Director, BMT Program) Royal Free Hospital, London, U.K.
 1990 to present Professor, Departments of Pediatrics and Medicine, University of Tennessee, Memphis, Tennessee
 1990 to present Member, Hematology/Oncology, and Director, Bone Marrow Transplant Program, St. Jude Children's Research Hospital, Memphis, Tennessee

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BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME	POSITION TITLE		
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EDUCATION (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)			
INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
University of Otago, Dunedin, New Zealand	MB ChB	1980	Medicine
Royal Australasian College of Physicians	FRACP	1987	Medicine
Royal Australasian College of Pathologists	FRCPA	1987	Hemat/Immunol
University of Otago, Dunedin, New Zealand	MD	1990	Hematology

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

- 1980-82 House Officer/Senior House Officer, Canterbury Hospital Board, Christchurch, New Zealand
- 1982-84 Medical Registrar, Canterbury Hospital Board, Christchurch, New Zealand
- 1984-85 Senior Registrar in Hematology, Canterbury Hospital Board, Christchurch, New Zealand
- 1986-89 Research Fellow/Honorary Lecturer, Royal Free Hospital, London, England
- 1989-91 Postdoctoral Research Associate, Department of Biochemistry and Hematology-Oncology, St. Jude Children's Research Hospital, Memphis, TN
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- 1991 to present Assistant Member, Division of Bone Marrow Transplantation, Department of Hematology-Oncology, St. Jude Children's Research Hospital, Memphis, Tennessee

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- Cordingley FT, Bianchi A, Hoffbrand AV, Reittie JE, Heslop HE, Vyakarnam A, Turner M, Meager A, Brenner MK. Tumour necrosis factor as an autocrine tumour growth factor for chronic B cell malignancies. *Lancet* i:969-971, 1988.
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- Heslop HE, Krance RA, Parham D, Brenner MK. Viruses and transplantation. *Encyclopedia of Virology* (in press).

Principal Investigator/Program Director (Last, first, middle):

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME	POSITION TITLE
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EDUCATION (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
Iowa State University	B.S.	1967	Zoology
University of Georgia	M.S.	1969	Biochemistry
University of Georgia	Ph.D.	1971	Biochemistry

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

- 1971-72 Postdoctoral Fellow, Oak Ridge National Laboratory, Tennessee
- 1972-73 Postdoctoral Fellow, American Cancer Society, Oak Ridge, Tennessee
- 1973-75 Staff Scientist, Oak Ridge National Laboratory, Tennessee
- 1975-79 Head, Immunobiology of Viral Carcinogenesis Section, Cancer Biology Program, NCI-Frederick Cancer Research Facility, Frederick, Maryland
- 1978-88 Associate, Department of Epidemiology, Division of Infectious Diseases, Johns Hopkins University, Baltimore, Maryland
- 1979-88 Associate, Department of Biochemistry, George Washington University Medical School, Washington, DC
- 1979-88 Head and Principal Research Scientist, Molecular Mechanisms in T-Cell Leukemogenesis Section, NCI-Frederick Cancer Research Facility, BRI-Basic Research Program, Frederick, Maryland
- 1988 to Present Professor, Department of Biochemistry, University of Tennessee, Memphis, Tennessee
- 1988 to Present Chairman, Department of Biochemistry, St. Jude Children's Research Hospital, Memphis, Tennessee

MOST RECENT PUBLICATIONS (192)

Keller JR, Ihle JN. Unique pathway of IL-3-driven hemopoietic differentiation. *J Immunol* 143:4025-4033, 1989.

Ihle JN, Morishita K, Parker DS, Bartholomew C, Askew D, Buchberg A, Jenkins NA, Copeland N, Weinstein Y. Mechanisms in the transformation of IL-3-dependent hematopoietic stem cells. *Curr Top Microbiol Immunol* 149:59-59, 1989.

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Matsugi T, Morishita K, Ihle JN. Identification, nuclear localization, and DNA-binding activity of the zinc finger protein encoded by the *Evi-1* myeloid transforming gene. *Mol Cell Biol* 10:1259-1264, 1990.

Ihle JN, Morishita K, Bartholomew C, Matsugi T, Askew D. Phenotypes and mechanisms in the transformation of hematopoietic cells. *Int J Cell Cloning* 8:130-146, 1990.

Morshita K, Parganas E, Douglass EC, Ihle JN. Unique expression of the human *Evi-1* gene in an endometrial carcinoma cell line: Sequence of cDNAs and structure of alternatively spliced transcripts. *Oncogene* 5:963-971, 1990.

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Goldwasser E, Pech N, Ihle J. Interleukin 3 can compensate for the deficiency in erythroid burst-forming cells in anemic mice of genotype *W/W^v*. *Exp Hematol* 18:936-939, 1990.

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CONTINUATION PAGE: STAY WITHIN MARGINS INDICATED

HUMAN GENE TRANSFER PROTOCOL

9303-040 Simons, Jonathan, Johns Hopkins Oncology Center, Baltimore, Maryland;
*Phase I Study of Non-Replicating Autologous Tumor Cell Injections Using
Cells Prepared With or Without Granulocyte-Macrophage Colony Stimulating
Factor Gene Transduction in Patients with Metastatic Renal Cell Carcinoma.*

Date of RAC Approval: 3/1/1993

Date of NIH Approval: 12/2/1993

Phase I Study of Non-Replicating Autologous Tumor Cell Therapy Using Cell Preparations with and without GM-CSF Gene Transduction in Patients with Metastatic Renal Cell Carcinoma

This phase I study in patients with advanced kidney cancer is undertaken with a view toward developing an effective means of treating disseminated cancers. The rationale for this trial is based on extensive pre-clinical studies in murine tumor models supplemented by *in vitro* explorations of the genetic modification of human renal cell cancer (RCC) cells and vaccine preparation.

Murine cancer cell lines genetically modified by the MFG retroviral vector containing cytokine genes have been screened for therapeutic anti-tumor activity. Screening included models of melanoma, sarcoma, renal cancer, lung cancer and colon cancer. Of 10 immunostimulatory molecules tested, only GM-CSF consistently showed superior antitumor efficacy in every cancer line tested. In both murine melanoma (B16) and murine RCC (RENCA), GM-CSF gene transduction enhanced the eradication of previously implanted tumors. Lethally irradiated, GM-CSF gene transduced murine cancer cells lost none of their potency. The genetically manipulated cells did not grow or cause significant toxicities at the site of administration.

With the MFG retroviral vector used in newly optimized *in vitro* culture conditions, over 30% of human RCC cells are transducible with a single transduction. In pilot studies, 20 of 22 consecutive primary cultures of RCC cells were transduced successfully. No drug selection was required. In these feasibility studies MFG retroviral transduction with the human GM-CSF gene increased baseline GM-CSF secretion by 10 to 20 fold over non-transduced RCC cells. Irradiation experiments defined a dose where patient derived RCC cells were rendered non-replicative. Fortunately, post-transduction GM-CSF secretion was not diminished by irradiation.

The overall objective of this phase I trial is to evaluate escalating doses of irradiated renal carcinoma cells with and without human GM-CSF gene transduction for safety of clinical administration and induction of antitumor immune responses. In order to distinguish toxicities related to cell dose from potential toxicities due to expression of GM-CSF, two clinical dose escalation studies are contained in one trial. In one set of patients, escalating doses of irradiated, autologous renal cell carcinoma cells (RCC) expanded in short-term culture and transduced with the human GM-CSF gene will be tested to determine the highest safely tolerated dose. Equivalent cell doses of similarly expanded, irradiated, non-transduced tumor cells will be tested in another set of RCC patients. Three specific primary study objectives are:

1. To evaluate the safety and tolerability of skin injections of cultured, irradiated, autologous RCC cells as well as similarly prepared RCC cells transduced with the human GM-CSF gene secreting the cytokine at 24-48 ng/10⁶ RCC cells/24 hr. The dose range of irradiated RCC cells under evaluation is 4X10⁶ to 1X10⁹.
2. To describe and quantitate acute clinical toxicities and long-term clinical toxicities of untransduced and GM-CSF gene transduced cell injections.
3. To assay both *in vitro* and *in vivo* the contribution of RCC cell GM-CSF gene transduction to the induction of specific antitumor immune responses relative to irradiated non-transduced RCC cells at equivalent cell doses.

NONTECHNICAL ABSTRACT

No consistently effective therapy exists for metastatic renal cell carcinoma (RCC). Currently, only 2% of patients with advanced RCC live five years following diagnosis. Interest in immunotherapy for RCC has been stimulated by the observation of spontaneous, short-term regressions of metastases without treatment in up to 1%-3% of patients. We have conducted extensive laboratory studies using a new strategy for inducing anti-tumor immune responses to mouse tumors including RCC. By inserting immunostimulatory genes into mouse RCC tumor cells, and injecting them under the skin, systemic antitumor immune responses have been reproducibly induced, resulting in eradication of small amounts of implanted tumor at distant sites. The Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) gene in these model studies conferred the most potent antitumor effects. Efficient introduction of this gene in the model cancer vaccine cells was accomplished with the retroviral vector MFG. This efficiency made feasible the generation of genetically engineered human cancer vaccines. Using the MFG vector, we were able to prepare kidney cancer cell vaccines from 20 of 22 cases in a preliminary study. Such transduced cells secrete 10 to 50 fold more GM-CSF than cultured, non-transduced RCC cells.

Lethal irradiation of the genetically engineered RCC cells did not diminish therapeutic effects of vaccine cells genetically engineered to secrete the GM-CSF gene in our mouse studies. Irradiation of tumor vaccines affords a measure of safety for human studies without compromising potential therapeutic efficacy. Otherwise, patients might have to be injected with live genetically engineered cancer cells for treatment. To develop this new strategy for the treatment of human cancer, safety of tumor vaccines produced by our procedure must be established in a phase I (toxicity) study.

The overall objective of this phase I study is to evaluate the safety and tolerability of RCC vaccine cell skin injections using a vaccine derived from a patient's RCC cells prepared either with or without human GM-CSF gene transfer. To help ensure safety, all tumor cell vaccines will be irradiated prior to injection. For evaluation of toxicities both from cancer vaccine cells alone, as well as from GM-CSF gene transfer, the trial will have two dose escalating studies within it. In one arm of the study, advanced kidney cancer patients will be treated with irradiated cells prepared without GM-CSF gene transfer. In the other arm of the study, patients will be treated with escalating doses of irradiated RCC cells transduced with the human GM-CSF gene. Patients will be randomly assigned to each arm. In all cases, surgical removal of the kidney will provide the source of the vaccine cells. The patients to be enrolled in this study will have greater than 1 billion cancer cells in their bodies as evidenced by the presence of visible metastases on X-rays. Unfortunately, the mouse RCC genetically engineered tumor vaccines were effective at eliminating up to about 10,000 cancer cells at distant sites. While it may be unlikely that the injections will benefit each patient enrolled in this trial with advanced disease, if safety can be established for these procedures, future studies involving larger number of RCC patients with occult metastases could be conducted.

PHASE I STUDY OF NON-REPLICATING AUTOLOGOUS TUMOR CELL INJECTIONS
USING CELLS PREPARED WITH OR WITHOUT GM-CSF GENE TRANSDUCTION IN
PATIENTS WITH METASTATIC RENAL CELL CARCINOMA

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15.0 REFERENCES

1.0 OBJECTIVES

This is a phase I toxicity study in patients with advanced renal cell carcinoma (RCC). The overall objective of this study is to evaluate a human tumor cell therapy with and without GM-CSF gene transduction for safety of clinical administration and induction of antitumor immune responses. In order to distinguish toxicities related to cell dose from potential toxicities due to expression of GM-CSF, two clinical dose escalation studies are contained in one trial. Escalating doses of lethally-irradiated, autologous renal cell carcinoma cells expanded in short-term culture and transduced with the human GM-CSF gene will be tested in one set of RCC patients to determine highest safely tolerated dose. Equivalent, escalating cell doses of expanded, irradiated, non-transduced RCC cells in another set of RCC patients will be evaluated for toxicities and determination of highest safely tolerated dose. Three primary objectives are:

1. To evaluate the safety of skin injections of cultured, lethally-irradiated, autologous RCC cells as well as similarly prepared RCC cells transduced with the human GM-CSF gene secreting the cytokine at 24-48 ng/10⁶ vaccine cells/24 hrs.
2. To describe and quantitate the acute toxicities (including maximum tolerated dose if one is approached) and long-term toxicities of GM-CSF gene transduced cell therapy and to distinguish clinical toxicities related to cell dosage alone from those related to high levels of GM-CSF expression.
3. To assay both *in vitro* and *in vivo* the contribution of RCC cell GM-CSF gene transduction to the induction of specific antitumor immune responses relative to irradiated non-transduced RCC vaccine cells.

Two secondary objectives are:

1. To seek preliminary evidence of therapeutic activity of injections of short-term cultured non-transduced irradiated autologous RCC cell injections.
2. To seek preliminary evidence of therapeutic activity of GM-CSF gene transduced autologous irradiated RCC cell injections.

2.0 BACKGROUND AND RATIONALE

2.1 CURRENT STATUS OF THERAPY FOR METASTATIC RENAL CELL CARCINOMA

MEDICAL THERAPY

New treatment is needed for metastatic renal cell carcinoma (RCC).^{1,2} The 5-year survival rate of Stage IV disease is 2%; median survival is about 18 months.^{1,2,3,4} For patients with supra-diaphragmatic extension of RCC into the vena cava (Stage III, T4b disease), and without radiographic evidence of distant metastases at surgery, the 5-year survival is equally dismal. To date chemotherapy and radiotherapy strategies have not shown any survival benefit.^{1,2,3,4}

Spontaneous, short-term regressions of RCC metastases have been reported in up to 1%-7% of RCC patients.^{2,5} This clinical observation suggests a role for host anti-tumor immunity in this disease, and fosters continued interest in immunotherapy. Interferon-alpha has been investigated as a treatment for metastatic RCC, with major responses observed in 10% to 21% of patients without clearly improved survival.⁵⁻⁹ Interleukin-2 (IL-2) has been approved by the FDA for the treatment of metastatic RCC after initial trials reported significant partial and complete response rates in up to one third of patients when used with LAK cells.^{10,11,12,13} Subsequent trials reported objective tumor response rates ranging from 0% to 35% of RCC patients with various schedules--with or without the infusion of LAK cells.¹⁰⁻²⁷ Complete responses, when they occur, may be durable for over 18 months. Associated with a complete response rate of 4% is a 4%-6% mortality rate from IL-2 treatment, according to the FDA approved package insert.²⁸ Most recently, a multi-institution phase II trial of rhuIL-2 and interferon alpha in metastatic RCC reported in July 1992 a complete response in 1 of 34 (3%) patients, toxicity in 4 of 34 (12%) patients requiring ICU admissions, and IL-2-related deaths in 2 of 34 (6%).²⁴ Investigational therapy thus remains a "standard of care" option for properly informed RCC patients. Phase I immunotherapy trials fall in this category, as well as phase I studies of novel cytotoxic drugs. Patients disinclined to be treated with IL-2, or participate in investigational trials are managed symptomatically.

SURGICAL THERAPY

Radical nephrectomy is the only curative treatment for RCC in Stage I, II, and III disease.^{1,2} Nephrectomy in advanced stage RCC patients (Stage III T4b and Stage IV) is used to palliate or forestall morbidity from complications of local tumor invasion into the great vessels or adjacent organs.^{1,2} Nephrectomy has not been shown in itself to prolong survival in Stage IV disease.^{1,2} In appropriate surgical candidates with Stage IV disease, however, nephrectomy has also been employed widely for preparation of

experimental immunotherapeutic agents. Examples include lymphokine activated killer cells (LAK therapy), tumor infiltrating lymphocytes (TIL therapy), and autologous lymphocyte transfusion (ALT therapy) which employ stimulator renal tumor cells harvested at surgery.² Nephrectomy has also been used in Stage III T4b and Stage IV patients to decrease tumor burden prior to new experimental schedules of IL-2 therapy.¹⁹

2.2 RATIONALE FOR CELL BASED IMMUNOTHERAPY FOR METASTATIC RENAL CELL CARCINOMA: PRECLINICAL DATA WITH MFG RETROVIRAL VECTOR

The transcendent rationale for tumor vaccine based immunotherapy of established cancer depends on the existence of tumor antigens capable of being recognized as foreign by the host immune system. The word "vaccine" is used in this context as the antigen source for activation of immune responses against established metastatic cancer, as opposed to prophylactic immunization. Recent evidence from a number of studies suggests that human cytotoxic T lymphocytes can recognize tumor-specific peptide antigens that arise either as a consequence of genetic alterations in human tumors, or induction in tumors of genes not expressed in normal adult tissues. The first human tumor-specific antigen recognized by human T cells was recently reported by Boon and colleagues, who identified a human melanoma specific antigen termed MAGE 1.³⁰

Active Immunotherapy of Cancer

Active immunotherapy involves the injection of autologous tumor cells to generate either a novel or an enhanced systemic immune response which will result in the eradication of metastatic sites. The reinjected tumor cells are generally altered in some way - by coinjection of adjuvants, by uv-irradiation, or by genetic manipulation - in an attempt to enhance their immunogenicity. Tumor vaccines have been used previously to treat patients with various solid tumors including RCC.³⁰⁻⁴⁵ Most studies have not compared vaccination strategies rigorously; unclear is which experimental variables exert significant effects on the resulting immune response. In the case of RCC, McCune and colleagues treated patients with irradiated tumor vaccines prepared immediately after surgery and administered weekly as intradermal injections. These cells were not expanded in primary culture. Doses ranged from 2.0 to 90 x10⁶ cells.³⁷ Eight of 16 patients, initially unreactive to a skin test with irradiated autologous tumor cells, converted to a positive delayed type hypersensitivity (DTH) reaction after therapy. Half of the DTH positive patients demonstrated prolonged survival associated with partial clinical responses.³⁷ No responses were observed if the DTH remained negative.³⁷ A significant advantage of "tumor vaccination" as active immunotherapy is that it is not reported to produce significant systemic toxicities, and can be delivered to outpatients. The most common toxicities reported previously are erythema, local pain, fever, and skin ulceration at the site of injection that generally resolve without intensive skin care in 1-3 weeks. More severe local reactions are related to the use of immune

adjuvants such as BCG.

Cell Therapy with Genetically Altered Tumor Vaccines

Recently, new approaches have been developed in laboratory animal systems that modify tumor cells genetically so that they express new antigens or secrete certain cytokines. These studies began with Lindemann and Kline who showed that vaccination with influenza virus infected murine tumor cells generated enhanced systemic immune responses against a challenge with the original wild-type tumor cells.⁴⁵

As newer techniques of gene transfer have been developed, infection with virus has been replaced with specific gene transfer systems in an attempt to regulate more carefully the nature of the genetic alteration in the tumor. A number of recent studies have used cytokine gene-modified tumor cells as tumor vaccines. Such studies have shown that transduction of some murine cancer cell lines with genes encoding IL-2, IL-4, interferon-gamma, IL-6, TNF-alpha, G-CSF, MCP-1, and IL-7 can lead to the rejection of the genetically modified cells by syngeneic hosts.⁴³⁻⁵⁸ Moreover, additional studies indicated that cells expressing inteferon-gamma, IL-2, TNF-alpha, or IL-4 also increase systemic immunity, since mice vaccinated with transduced cells reject a subsequent challenge by wild-type tumor cells, and in some cases, reject pre-existing tumor.⁵⁰

Based on these pre-clinical studies, several investigators have received approval to initiate clinical protocols which involve the injection of genetically modified tumor cells into patients with advanced cancer. Investigators at the National Cancer Institute, for example, received approval to inject live autologous tumor cells engineered by retroviral mediated gene transfer to express either TNF-alpha or IL-2. Investigators at Memorial Sloan Kettering Cancer Center have recently received approval for clinical studies involving tumor cells engineered to express IL-2 in melanomas and renal cell carcinomas.

The protocol described below, however, differs from those previous protocols in several ways. First, irradiated tumor cells, rather than live cells, will be administered to patients. This feature of the protocol reduces the risk of toxicities associated with the insertion of genetic material into the chromosomal DNA of the tumor cells. Second, low passage cultures of freshly-explanted autologous tumor will be the targets for genetic modification, rather than established tumor cell lines. This feature of the protocol is designed to preserve any antigenic heterogeneity that may exist in the tumor, thereby maximizing the chance for eliciting a broad systemic anti-tumor immunity in vaccinated patients. Finally, tumor cells will be engineered to express GM-CSF, rather than TNF-alpha or IL-2, since our pre-clinical studies suggest that GM-CSF may be more potent than other gene products in inducing systemic anti-tumor immunity.

Preclinical Studies Involving Irradiated, Genetically Modified Tumor Cells

The decision to employ irradiated tumor cells expressing GM-CSF for our clinical protocol was based upon collaborative studies between Dr. Mulligan's laboratory at the Whitehead Institute and M.I.T. and Dr. Pardoll's laboratory at Johns Hopkins. One rationale for these studies was to assess the relative potency of the many different molecules previously identified as possessing immunostimulatory activity, and to identify new gene products capable of inducing systemic antitumor immunity. No previous study had compared the activity of different gene products in a single tumor model, nor examined the activity of a single gene product in multiple tumor models. A second rationale was the need to understand, in a general way, some of the mechanisms underlying the immunostimulatory activity of cytokines and other gene products.

The important finding that irradiated, transduced tumor cells were as capable of inducing potent systemic anti-tumor immunity as live, transduced cells was made through an early series of experiments in which the effects of the expression of a variety of different cytokines and adhesion molecules upon the tumorigenicity of the mouse melanoma cell line, B16, was examined. To facilitate the transfer of a variety of gene products into tumor cells, a recombinant retrovirus vector, MFG, was utilized (see Appendix 14.B). Studies from Dr. Mulligan's laboratory had shown that this vector consistently yielded high titers of helper-free recombinant virus, and high levels of the desired gene product in infected cells (Appendix 14.A.3). Ten different molecules were examined for their effect upon the tumorigenicity of B16 cells. These included IL-2, IL-4, IFN-gamma, and TNF-alpha, as well as a number of gene products not previously examined (IL-5, IL-6, IL-1RA, CD2, ICAM-1, and GM-CSF). Of the ten molecules tested, only IL-2 promoted the complete rejection of B16 cells. Several of the other molecules caused a partial delay in tumor growth. Expression of a number of other gene products, including TNF, IL-6, and GM-CSF, actively induced systemic toxicities, due to the progressive growth of tumor cells expressing those products.

Although IL-2 transduced B16 cells were effectively rejected, they did not protect animals from a subsequent challenge with non-transduced cells. Since only IL-2 expression resulted in rejection of B16 cells, challenge experiments were next performed using cells doubly infected with an IL-2 expressing virus and an additional construct. It was through this analysis that GM-CSF was first identified as a powerful inducer of systemic anti-tumor immunity. Since B16 cells expressing GM-CSF alone had been shown to grow progressively, it was possible that in the case of cells expressing both IL-2 and GM-CSF, IL-2 expression served primarily to cause rejection of the vaccinating cells. This was shown to be the case, since B16 cells that were expressing GM-CSF alone, but irradiated with 3500 rads to prevent their outgrowth in vivo, stimulated potent systemic anti-tumor immunity (Figure 1A). Non-transduced, but irradiated B16 cells showed little or no stimulation of

systemic immunity. Subsequent studies have shown that irradiated cells, while unable to replicate, are metabolically active, and express inserted genes for over one week in vitro.

Based on the above findings, experiments were then performed to compare the relative potency of irradiated tumor cells expressing the ten molecules previously examined using live, transduced tumor cells. As shown in Figure 1B, GM-CSF was the most potent molecule tested, IL-4 and IL-6 possessed some activity, and notably, IL-2, IFN-gamma, and TNF-alpha, showed no activity. These results provided the major rationale for selecting GM-CSF, rather than other molecules, for our clinical studies.

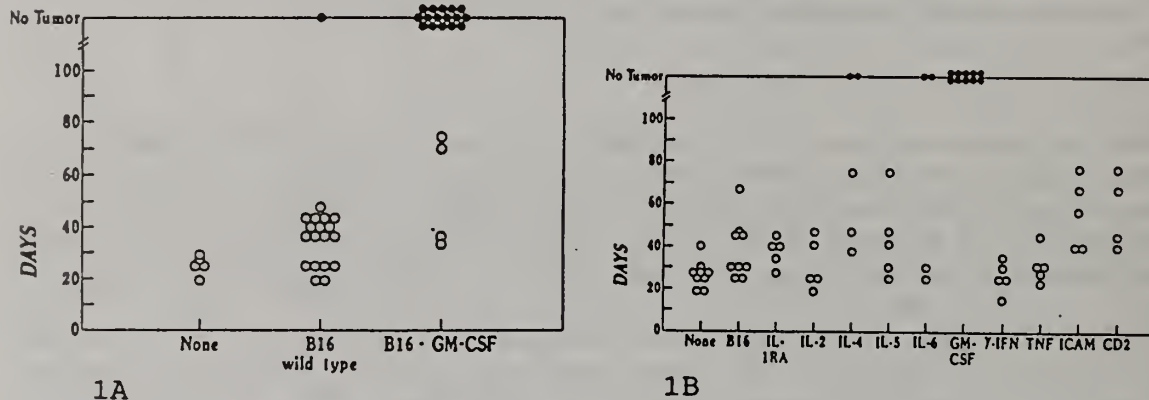


Figure 1A: Irradiated B16 Melanoma Cells Expressing GM-CSF Confer Potent Systemic Antitumor Effects. Mice were vaccinated with 5×10^6 irradiated (3,500 rads) GM-CSF transduced or non-transduced B16 cells. o animals displaying growth of tumor challenge. • animals protected from tumor challenge.

Figure 1B: Vaccinations with irradiated B16 melanoma cells expressing cytokines and adhesion molecules. Mice were vaccinated with 5×10^5 cells irradiated with 5000 rads. Animals were challenged 7 days later with 1×10^6 live, wild-type B16 tumor cells.

Immunologic analysis of the response generated by GM-CSF transduced tumor cells indicated that systemic immunity was dependent on both CD4+ and CD8+ T cells and was long lived, since mice challenged several months after vaccination continued to be protected (Appendix 14.A.4).

Experiments were performed to determine the importance of immunizing cell dose on systemic protection. As shown in figure 2, immune protection against a challenge with live wild type tumor was highly sensitive to the total inoculum of vaccinating cells; increasing doses of GM-CSF transduced tumor cells resulted in enhanced systemic antitumor immunity. These results suggest that higher immunizing cell doses will have greater therapeutic efficacy in human cancers.

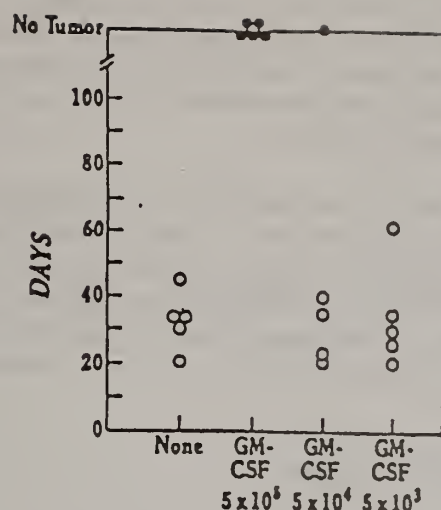


Figure 2. Vaccinations with irradiated GM-CSF transduced melanoma cells. Mice were vaccinated with varying numbers of irradiated (5000 rad) GM-CSF transduced tumor cells. Animals were challenged with 5×10^5 live, wild type B16 tumor cells 7 days later. o animals dying of tumor challenge • animals protected against tumor challenge.

Dilution experiments in which irradiated non-transduced cells were mixed with irradiated GM-CSF transduced cells indicated that not all of the cells needed to produce GM-CSF to induce a full systemic immune response. Indeed, full anti-tumor immunization potential was apparent over a reasonably wide range of GM-CSF concentrations. These dilution experiments indicated that an average production rate of approximately 36 nanograms/ 10^6 cells/24 hours was sufficient to induce the maximum protective immune response possible (Table 2).

Table 2

Effect of average GM-CSF production rate.

Avg. GM-CSF production ^a	# mice tumor free 30 days after challenge ^b
360 ng/ 10^6 cells/24 hr	7/10
120 ng/ 10^6 cells/24 hr	7/10
36 ng/ 10^6 cells/24 hr	7/10
12 ng/ 10^6 cells/24 hr	3/10
3.6 ng/ 10^6 cells/24 hr	1/10
0 ng/ 10^6 cells/24 hr	0/10

^a Mice were vaccinated with varying numbers of irradiated GM-CSF transduced cells mixed with irradiated nontransduced cells such that the total number of immunizing cells was held constant at 1×10^7 and average GM-CSF production per cell were varied between 0 and 360 ng/ 10^6 cells/24 hr.

^b Mice were challenged subcutaneously with 1×10^6 live nontransduced B16 cells 2 weeks after vaccination.

These results provide the basis for the dose escalation design of this trial: lethally-irradiated RCC vaccine cell dose is escalated at a fixed range of average GM-CSF cytokine production per cell. The range of GM-CSF secretion chosen produces maximal systemic antitumor immune responses in the preclinical models. The importance of sustained, local GM-CSF production achieved by gene transduction is underscored by the failure of mixing soluble recombinant GM-CSF with non-transduced irradiated tumor vaccine cells to generate any measurable antitumor immunity.

The results also underscore the importance of assessing independently the toxicity that may be attributable to cell dose alone from that associated with GM-CSF expression. If, for example, our clinical studies suggest that the highest dose of non-transduced cells is well tolerated, yet the highest dose of transduced cells is not, it may be possible to obtain maximum benefit from the therapy by simply reducing the proportion of transduced cells at the highest cell dose.

Studies in Other Murine Tumor Models

Several well characterized murine tumor models were used to examine the generality of the host response to vaccination with irradiated, GM-CSF expressing tumor cells. These tumors included CT26, a colon carcinoma; CMS 5, a fibrosarcoma; RENCA, a renal cell carcinoma; and WP 4, another fibrosarcoma. In these studies animals were vaccinated with irradiated wild-type tumor cells or with irradiated tumor cells transduced with GM-CSF. Three weeks later, the vaccinated animals were challenged with live, wild-type tumor cells.

In contrast to the experience with the B16 model (Figure 1), irradiated tumor cells possessed potent immunogenic activity (Figure 3). These results may provide at least a partial explanation for the ability to detect the immunostimulatory activity of a number of cytokines in these models, but not in B16.

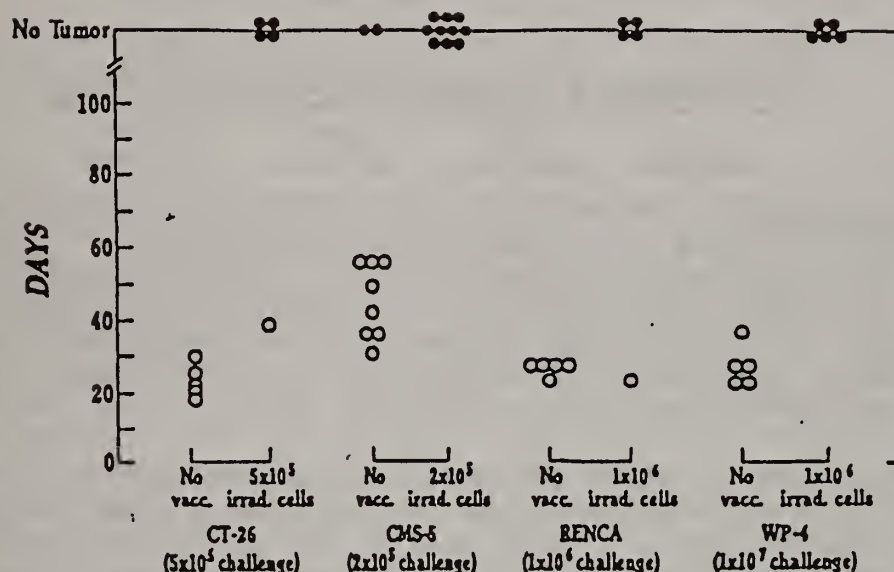


Figure 3. Immunogenicity of irradiated, non-transduced murine tumor cells. Animals were vaccinated with irradiated, wild-type tumor as indicated. Three weeks later they were challenged by the subcutaneous injection of live, wild-type tumor cells. o animals displaying growth of tumor challenge. • animals protected against challenge.

Since irradiated wild-type tumor cells possessed significant immunogenic activities, it was not a simple matter to examine the added effects of GM-CSF or other cytokines. However, a lengthy exploration of vaccine cell dose and tumor cell challenge size in each tumor system resulted in defining experimental conditions which permitted valid comparisons of the immunogenic potency of irradiated, non-transduced tumor cells with similarly prepared cells secreting GM-CSF. Although the relative efficacy of GM-CSF secreting cells varied somewhat, in all models examined they were more potent stimulators of a systemic immune response than their counterpart, non-transduced cells (Figure 4).

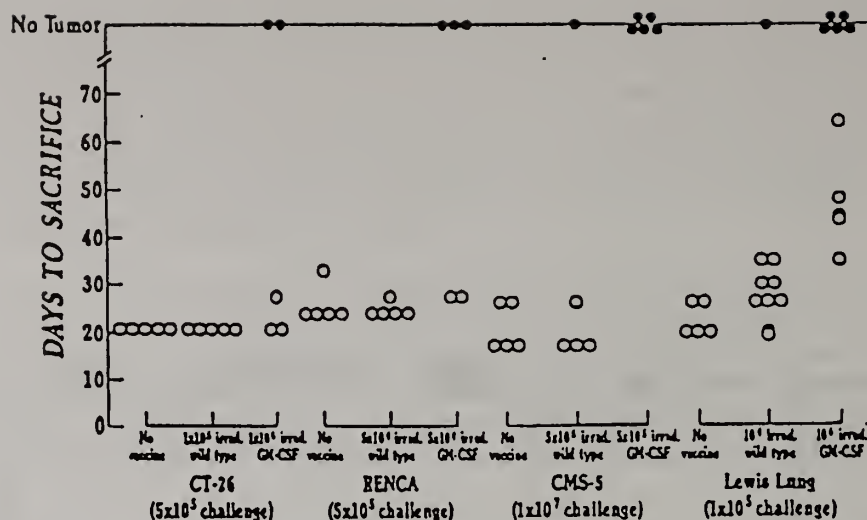


Figure 4. Irradiated, GM-CSF secreting tumor cells stimulate increased systemic immunity relative to their non-transduced counterparts. Mice were inoculated with irradiated, non-transduced tumor cells or irradiated tumor cells secreting GM-CSF. Three weeks later, they were challenged with live wild-type tumor. $\circ$ animals displaying growth of tumor challenge. $\bullet$ animals protected against tumor challenge. Model tumor lines: CT-26(colon ca), RENCA(renal ca), CMS-5(sarcoma), Lewis Lung (lung ca).

The therapeutic utility of GM-CSF transduced tumor cell vaccines to cause rejection of pre-established tumors was explored in several model systems in a similar manner. In each model system, the size of the tumor to be treated (the time of treatment after tumor inoculation) was an important determinant of the outcome. Tables 6 and 7 show data from representative experiments. The results in treating small sized pulmonary metastases (Table 7) seems especially instructive to the clinicians who commonly encounter this in RCC patients.

Table 6

Treatment of mice bearing small pre-existing deposits of established tumor with GM-CSF gene transduced tumor cells

Tumor System	Number of Animals Tumor Free 100 days After Treatment	
B16 melanoma ^a	5x10 ⁴ tumor cells implanted	1x10 ⁵ tumor cells implanted
No treatment control	1/5	0/5
Irradiated wildtype	0/5	0/5
Irradiated+GM-CSF gene	4/5	2/5
Renca RCC ^b	5x10 ³ tumor cells implanted	1x10 ⁴ tumor cells implanted
No treatment control	0/10	1/10
Irradiated wildtype	5/10	3/10
Irradiated+GM-CSF	10/10	9/10

^a For the B16 tumor, C57BL/6 mice were treated with 1x10⁶ live wildtype, irradiated wildtype, or irradiated GM-CSF gene transduced cells 2 days after injection of the live tumor.

^b For the Renca tumor, Balb/c mice were treated with 1x10⁶ live wildtype, irradiated wildtype, or irradiated GM-CSF gene transduced tumor cells 6 days after injection of the live tumor.

Table 7

Treatment of B16 melanoma lung metastases with irradiated GM-CSF transduced tumor cells.

Treatment	mean # lung tumors/mouse Exp 1 ^a	#mice tumor free Exp 2 ^b
None	32	11.5
Irradiated wild type	32	5.5
Irradiated GM-CSF	12	0.2

^a C57BL6 mice were injected in the tail vein with 2×10^4 live wild type B16 melanoma cells. 3 days later they were injected subcutaneously with irradiated wild type or irradiated GM-CSF transduced B16 melanoma cells. 28 days later, lungs were removed and examined both macroscopically and microscopically.

^b C57BL6 mice were injected in the tail vein with 1.5×10^4 live wild type B16 melanoma cells. 3 days later they were injected subcutaneously with 1×10^6 irradiated wild type or irradiated GM-CSF transduced B16 melanoma cells. 28 days later, lungs were removed and examined both macroscopically and microscopically.

The general conclusion from the results of therapeutic experiments in these murine models is that it is possible to cure animals burdened with a relatively small amount of pre-established tumor which could be equated with the micro-metastatic disease that is encountered frequently in the clinic. If so, it appears unlikely that radio-graphically detected metastatic disease in humans (tumor burden $>10^9$ cells) will be eradicated by the highest vaccine doses used in this trial. Therefore, if safety and immunogenic potency is established in this trial, studies of efficacy might best be pursued with adjuvant therapy for patients with minimal tumor burdens (e.g., following surgical resection) who are at high risk of recurrence because of the presence of micro-metastatic tumor. In the U.S., over one third of patients undergoing radical nephrectomy for RCC with curative intent relapse from micro-metastatic disease.²

2.3 FEASIBILITY OF PRODUCING A GM-CSF SECRETING RCC TUMOR VACCINE

Feasibility of establishing and transducing primary human tumor cultures.

The methods proposed are adequate to establish and transduce each patient's RCC cells following nephrectomy. Before submitting this protocol to the Johns Hopkins Joint Committee on Clinical Investigation, feasibility studies were conducted within the clinical research setting of the Johns Hopkins Oncology Center and Department of Urology where the trial is to take place. Of 29 consecutive RCC specimens received at the time of surgical excision, 25 were established as primary cell cultures. Among the 4 specimens not established were two benign tumors, one tumor contaminated at the time of resection, and 1 oncocytoma. Of the 25 tumors that were established as primary cultures, 22 of these patients had histologic subtypes that would have made them eligible for this trial. The 3 of 25 patients that would probably be ineligible for our trial had oncocytomas. Patients with oncocytomas will not be eligible for this study because these tumors have a benign course, and are rarely discovered as stage III or IV disease. Finally, of the 22

"eligible patients" in this mock-up, 20 (91%) could have received the GM-CSF-secreting tumor vaccine based on our ability to expand and transduce the cells in culture.

In these studies, 7 consecutive renal cell tumors were initially expanded and transduced with the MFG vector carrying the Lacz marker gene. The last *in vitro* passage obtained and the percentage of cells expressing beta-galactosidase activity is shown in table 8. The percentage of Lacz expression among all of the optimally transduced tumor cells ranged from 40 to 100%.

Feasibility of vaccine production at Somatix Therpy Corporation.

We have found that it is also feasible to sterily transport on wet ice overnight to Somatix in Alameda, California, in a thermally secure container, the excised tumors that have first been mechanically dissociated into 0.5 to 1 cm fragments. These fragments can be subsequently digested into single cell suspension, and expanded and transduced with the MFG vector carrying the human GM-CSF gene. The results of enzymatic digestion and *in vitro* expansion are shown in Table 9A. Eight consecutive renal cell tumors were evaluated. A range of 6×10^6 to 1×10^9 total viable cells with a mean of 2.2×10^8 cells were obtained from the digested tumors. The total number of passages of expanded cells ranged from P2 to P5.

Table 9B shows the results of transduction of 5 renal cell tumors with the MFG vector carrying the human GM-CSF gene. All transductions were performed at Somatix using retroviral supernatants that were produced using the cell cube system described in Appendix 14.B. This system routinely produces retroviral supernatants with titers at least 10 fold higher than what had been obtained when supernatants were collected from cells grown in traditional tissue culture flasks. Furthermore, these supernatants can be frozen for at least one month at -70°C with minimal loss of virus titer. Therefore, large quantities of retroviral supernatants can be titered and stored, providing greater consistency between transductions. All 5 of the renal tumors transduced with different batches of GM-CSF retroviral supernatant were shown to express between 24 and $57.6 \text{ ng}/10^6$ tumor cells/24 hours, as determined by ELISA. This is between 10 and 50 fold greater levels of GM-CSF than endogenously produced and measured in untransduced patient derived controls. Based on our murine data, this level of expression is within the range that is expected to be therapeutic in patients. The procedures used for processing the human tumor cells, establishing primary cultures, and transducing the cells with the retroviral vector can be found in Appendix 14.C.

Determining tumor cell purity of primary renal cell cultures.

Determining the relative purity of the cancer cell population that is expanded in the primary cultures is a difficult problem because renal tumor-specific markers for quantitation are not yet developed. Several criteria can be used to evaluate these cultures for overwhelming presence of cancer cells.

The most important determination of cell purity is histologic evaluation. Malignant renal tumors have histologic characteristics that distinguish them as malignant cells of epithelial origin. Their cytoplasm often contains many granules (granular cell) or lipid deposits (clear cell) that differentiates them from cells of mesenchymal origin such as fibroblasts. Although it is sometimes difficult to distinguish normal from malignant cells in culture, it is unusual to have normal contaminating epithelial cells within the excised mass. Furthermore, when normal renal epithelium growth is compared with malignant renal epithelium growth *in vitro*, the normal exhibits contact inhibition whereas the malignant does not.

Besides normal renal cells of epithelial origin, other cells of non-epithelial origin can also contaminate the primary tumor cell cultures. Lymphocytes, macrophages and red blood cells are often present in the initial cultures especially when the tumor is necrotic. But these cells do not propagate under the renal tumor growth conditions. Fibroblasts are a concern in many types of primary tumor cultures, but not a great concern in renal tumors due to the minimal amount of reactive tissue present at the site of these tumors. However, histochemical staining is performed routinely with anticytokeratin antibodies to distinguish cells of epithelial origin from cells of mesenchymal origin. Ten consecutive primary renal tumor were evaluated for cytokeratin expression at the time of enzymatic digestion and again after *in vitro* passage. Positive cytokeratin staining ranged from 40 to 95% initially, and increased to between 60 and 100% by *in vitro* passage 4, with the majority of cultures demonstrating greater than 80% cytokeratin expression (Table 10). These results strongly confirm that all of the primary cultures are composed of a majority of cells of epithelial origin.

Establishing the safety of autologous GM-CSF-secreting human tumor vaccines.

Also critical to the development of a human cytokine-secreting tumor vaccine is safety after re-injection of the tumor cells back into the patient. We therefore evaluated the primary human renal cell tumor explants for *in vitro* growth and GM-CSF expression following lethal doses of irradiation. Initially 5 fresh human renal tumor cultures were evaluated for the optimal dose above which none of the tumor cells proliferated in culture. The results are shown in Table 11. A radiation dose of 15,000 rads or greater resulted in 100% inhibition of proliferation by day 3 following radiation. All of the tumor cells in culture were noted to die by day 28 following irradiation. Next we evaluated GM-CSF secretion by the transduced tumor vaccine cells after receiving 5,000, 10,000 or 15,000 rads, and compared the results with GM-CSF production by the unirradiated vaccine cells. The results for one renal tumor vaccine are shown in Figure 5. Despite even 15,000 rads, all of the tumors evaluated were able to secrete at least as much human GM-CSF as the unirradiated, transduced vaccine cells when comparisons were made 1 day and 4 days after irradiation. Therefore, it is possible to inhibit proliferation of the RCC vaccine cell population without affecting expression of the GM-CSF gene. Other pre-clinical toxicity data is presented in Appendix 14.A.2.

Feasibility of storing the genetically-altered tumor vaccines in liquid nitrogen until the day of vaccination.

As outlined in the treatment plan, patient tumor specimens will be processed, expanded and transduced at Somatix which is located in Alameda, Ca. The genetically-altered tumor vaccine cells will then be frozen and sent back to Johns Hopkins Oncology Center where they will be stored in a liquid nitrogen freezer until the day that the patient is vaccinated. To determine the efficacy of the freshly thawed, genetically-altered tumor vaccine, mice were treated with freshly thawed B16 melanoma cells that had been genetically-altered to secrete GM-CSF, frozen, and stored in a liquid nitrogen freezer until the day of vaccination (Figure 6). On day 0, 10 mice per group were vaccinated with either 1×10^6 GM-CSF secreting vaccine cells grown in culture, 1×10^6 GM-CSF secreting vaccine cells freshly thawed from the liquid nitrogen freezer, or 1×10^6 untransduced B16 melanoma cells. All vaccines were irradiated with 5000 rads prior to the subcutaneous vaccination in the left flank. On day 14 following vaccination, mice were challenged with 1×10^5 B16 wild-type tumor cells in the right flank. Mice were evaluated twice a week for the development of tumor. None of the mice treated with either the freshly thawed or *in vitro* grown vaccine cells developed tumors. 70% of the mice treated with the 1×10^6 irradiated wild-type vaccine cells developed tumors. These results were confirmed in a second experiment. This data shows that freshly thawed and irradiated genetically-altered tumor vaccine cells are at least as effective as the *in vitro* grown and irradiated genetically-altered tumor vaccine cells.

TABLES 8,9A,9B,10,11 AND FIGURES 5 AND 6 FOLLOW ON PAGES 18-23.

Table 8. Feasibility of short-term *in vitro* expansion and transduction of fresh renal tumor explants. Fresh renal tumor explants from 7 different patients have been grown *in vitro* over a 4-6 week period and have been transduced with the E.Coli Beta-galactosidase marker gene between passages 1 and 5. All of the tumors listed in this table were grown and transduced at Johns Hopkins Oncology Center. Of note, *in vitro* expansion and transduction efficiency does not seem to be determined by the histologic subtype or nuclear grade of the explanted renal tumor.

RCC CODE	LAST IN VITRO PASSAGE	HISTOLOGY	TRANSDUCTION EFFICIENCY
RCC 3.8.91	P6	Papillary + clear cell and granular cell features	100.0%
RCC 5.1.91	P7	Grade II Clear cell	100.0%
RCC 6.25.91	P4	Granular cell	78.0%
RCC 7.9.91	P7	Clear cell, Grade II	64.0%
RCC 10.14.91	P5	Sarcomatoid	59.3%
RCC 10.14.91 VC	P4 Then transformed to a cell line	Sarcomatoid	86.4%
RCC 10.25.91	P5	Grade II, clear cell	39.7%

Table 9A. Feasibility of transporting fresh human tumor explants to Somatix for the production of the genetically-altered GMCSF-secreting tumor vaccine. Since 5/22/92, 8 renal cell carcinomas were transported to Somatix for in vitro expansion and transduction. The first 3 tumors (RCC 5.22.92, 6.29.92, 7.20.92) were first mechanically dissociated and digested to a single cell suspension at Johns Hopkins Oncology Center, and then transported frozen on dry ice. The subsequent 5 tumors (8.28.92, 9.14.92, 9.15.92, 11.30.92, 12.10.92) were initially mechanically dissociated into 0.5 cm fragments, then transported on wet ice overnight to Somatix. The tumor fragments were enzymatically digested approximately 18 hours later at Somatix. The total # of viable cells and the last in vitro passage achieved are recorded below. The % viability after enzymatic digestion is recorded in parenthesis.

TUMOR CODE	# VIABLE TUMOR CELLS (% VIABILITY)	LAST PASSAGE OBTAINED	HISTOLOGY
RCC 5.22.92	3.3 X 10 ⁷	P3	Multicystic, grade III, with oncocytic features
RCC 6.29.92	9.0 X 10 ⁶	P5	Clear cell, grade II
RCC 7.20.92	11.2 X 10 ⁸	P3	Grade III
RCC 8.28.92	1.2 X 10 ⁸ (67%)	P5	Grade II AND III Granular cell
RCC 9.14.92	2.2 X 10 ⁸ (88%)	P2	Granular cell with clear cell features
RCC 9.15.92	1.8 X 10 ⁷ (60%)	P4	Grade I and II
RCC 11.30.92	6.8 X 10 ⁶ (87%)	P3, still growing	Oncocytoma
RCC 12.10.92	2.8 X 10 ⁸ (86%)	P3, still growing	Grade II with oncocytic features

Table 9B. Feasibility of short-term *in vitro* expansion and transduction of fresh renal tumor explants with the GM-CSF gene after transport to Somatix. Fresh renal tumor explants from 5 different patients have been grown *in vitro* over a 4-6 week period and have been transduced with the human GM-CSF gene. All 5 patient samples have been grown and transduced at Somatix. The transduced cells were assayed by ELISA for GM-CSF secretion *in vitro*. The ELISA assay has a sensitivity limit of 1.5 picograms per ml. Secretion of an active GM-CSF protein was confirmed by bioassay which evaluates proliferation of a GM-CSF-dependent cell line (TF-1 cells). Transduction efficiency and GM-CSF expression does not appear to be affected by histologic subtype.

TUMOR CODE	GM-CSF EXPRESSION POST- TRANSDUCTION (ng/10 ⁶ cells/24 hours)	LAST IN VITRO PASSAGE	HISTOLOGY
RCC 7.1.91	24	P13	Sarcomatoid
RCC 7.9.91	54.7	P7	Clear cell, grade II
RCC 5.22.92	34.32	P3	Multicystic, Grade III, with oncocyctic features
RCC 6.29.92	35.05	P5	Clear cell, Grade II
RCC 7.20.92	57.6	P3	Clear cell, Grade III

Table 10. Characterization of the fresh human tumor explants for cytokeritin production and MHC class I and II levels. Fresh renal tumor explants from 10 patients were analyzed just after enzymatic digestion for cytokeritin production by flow cytometry. Six of these tumors were also assayed again after transduction by histochemical evaluation. The *in vitro* passage at which the cells were analyzed is in parenthesis. The histochemical staining is employed post-transduction in order to conserve vaccine cells. MHC class I and II levels were evaluated for 4 of the tumors evaluated at Somatix. An additional 13 renal cell tumors were also evaluated at JHOC for MHC class I and II expression. All 13 tumors expressed high levels of MHC class I and either low or no MHC class II molecules (data not shown). All tumors were assayed for MHC antigen expression by flow cytometry using fluorocein-labeled monoclonal antibodies.

TUMOR CODE	% OF CELLS PRODUCING KERITIN PRE-DIGESTION	% OF CELLS PRODUCING KERITIN AT TIME OF TRANSDUCTION	MHC CLASS I/II LEVELS	HISTOLOGY
RCC 7.15.91	50		High/none	Sarcomatoid
RCC 5.22.92	95		High/none	Multicystic, grade III with oncocyctic features
RCC 6.29.92	95			Clear cell Grade II
RCC 7.20.92	50	100	99%/0	Clear cell Grade III
RCC 8.28.92	60	80 (P2)	100%/0	Grade II/III Granular cell
RCC 9.14.92	87			Granular cell with clear cell features
RCC 9.15.92	50	95 (P4)		Grade I/II
RCC 9.22.92	40	100		Granular cell
RCC 9.24.92		100 (P3)		Papillary, grade II
RCC 10.1.92	40	100 (P2)		Clear Cell Grade III

Table 11. Evaluation of the range of irradiation doses that result in 100% inhibition of proliferation of renal cell tumors *in vitro*. 5 fresh renal tumor cells of varying *in vitro* passages between 1 and 5, were irradiated with a range of doses from 5,000 to 40,000 rads and plated at a density of 1×10^5 cells per 25 cm² flask. Cells were scored daily for *in vitro* growth and cell death. All of the renal cell tumors were evaluated prior to transduction.

TUMOR CODE	POST-IRRADIATION EVENTS IN VITRO	RANGE OF IRRADIATION DOSES	HISTOLOGY
RCC 7.18.91	<u>DAY 5</u> - Slow growth all doses <u>DAY 9</u> - 20% Cell death at >15,000 rads <u>DAY 17</u> - 50% Cell death at all doses <u>DAY 28</u> - 100% Cell death all doses	5000-30000 rads	Papillary, grade II
RCC 10.14.91	<u>DAY 3</u> - Enlarged cells at >15,000 rads <u>DAY 7</u> - Enlarged cells, no growth all doses, 10% death >14,000 rads <u>DAY 14</u> - 50% Cell death all doses <u>DAY 27</u> - 100% Cell death at all doses	5000 - 23000 rads	Sarcomatoid
RCC 11.4.91	<u>DAY 3</u> - No growth, enlarging cells >26,000 rads <u>DAY 4</u> - Slow growth, enlarging cells > 12,000 rads <u>DAY 6</u> - 10% Cell death >12,000 rads <u>DAY 19</u> - >90% Cell death at all doses	12000 - 33000 rads	Grade II Oncocytoma
RCC 6.23.92	<u>DAY 4</u> - >15% Cell death at all doses <u>DAY 15</u> - >90% Cell death at all doses	23000 - 39000 rads	Grade II, Cystic
RCC 6.29.92	<u>DAY 3</u> - >15% Cell death at all doses DAY 13 - >90% Cell death at all doses	23000 - 39000 rads	Clear cell, Grade II

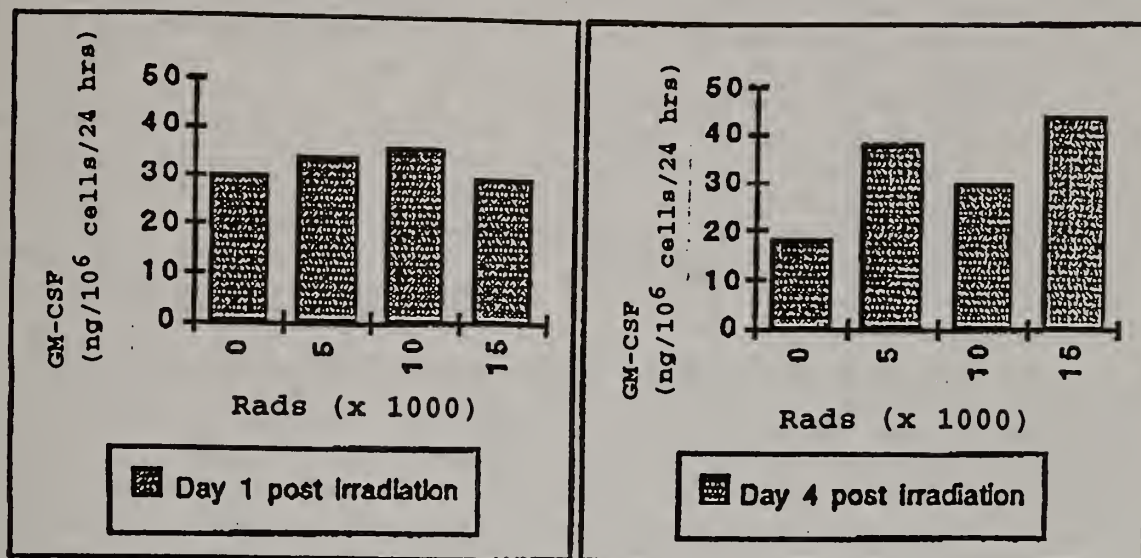


Figure 5. Evaluation of the effects of irradiation on GM-CSF production by transduced renal carcinoma cells. Transduced RCC vaccine cells were either irradiated with a total dose of 5,000, 10,000 or 15,000 rads, or left untreated, and subsequently cultured for 4 days. Supernatants were collected every 24 hours and replaced with fresh tumor media. The 24 hour supernatants for days 1 and 4 were assayed for GM-CSF production by ELISA. The results revealed that irradiated, transduced cells produced at least as much GM-CSF as did the non-irradiated controls, demonstrating that the lymphokine secreting capacity of the vaccine cells was not impaired by radiation treatment.

Evaluation of thawed vs in vitro grown GM-CSF tumor vaccines

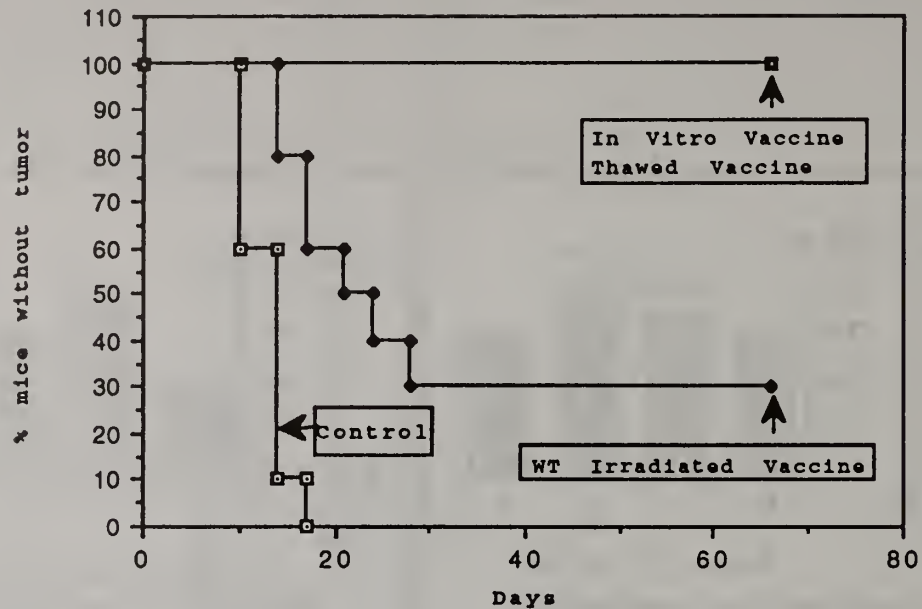


Figure 6. Feasibility of vaccinating with a freshly thawed genetically-altered tumor vaccine. Mice were treated with freshly thawed B16 melanoma cells genetically-altered to secrete GM-CSF and frozen down and stored in a liquid nitrogen freezer until the day of vaccination. On day 0, 10 mice per group were vaccinated with either 1×10^6 GM-CSF secreting vaccine cells grown in culture, 1×10^6 GM-CSF secreting vaccine cells freshly thawed from the liquid nitrogen freezer and washed twice with Hank's balanced salt solution, or 1×10^6 untransduced B16 melanoma cells. All vaccines were irradiated with 5000 rads prior to subcutaneous vaccination in the left flank. On day 14 following vaccination, mice were challenged with 1×10^5 B16 wild type melanoma cells in the right flank. Mice were evaluated twice a week for the development of tumor. These results were confirmed in a second experiment.

Other Preclinical Data

The murine tumor studies summarized above are detailed in the manuscript by Dranoff et. al., PNAS in press (Appendix 14.A.3) and in appendix 14.A.4. In addition, data relating to the serum pharmacokinetics of GM-CSF and toxicity studies involving mice injected with GM-CSF transduced kidney tumor cells can be found in Appendices 14.A.1 and 14.A.2. A protocol for preparation of human RCC tumor vaccines was developed on the basis of the above studies and is described in detail in Appendix 14.C.

3.0 STUDY DESIGN AND TREATMENT PLAN

3.1 OVERVIEW OF RESEARCH OBJECTIVES

The main objective of this trial is to evaluate the safety of RCC cell injections prepared with and without GM-CSF gene transduction. Emphasis is placed on documenting the adverse effects of vaccinations on tissues at or near the site of injection, as well as acute and longer-term systemic toxicities. As discussed in the previous section, such an independent assessment of maximum tolerated cell and cytokine dose will be critical for the selection of an optimal RCC cell preparation and schedule of administration for phase II trials of therapeutic efficacy. The two armed study design is employed specifically to allow distinction of toxicities due to cell administration from those due to local GM-CSF production by the transduced tumor cells.

Also critical is evaluation of whether or not tumor vaccines non-transduced or transduced with the GM-CSF gene can induce a specific, antitumor immune response in patients. The parallel dose escalation arms in the study design may additionally aid in the evaluation of the contribution of GM-CSF gene transfer to antitumor immune response induction. A number of points bare on this issue. First, at higher cell doses irradiated tumor vaccines have significant anti-tumor immunogenicity in some pre-clinical tumor models without GM-CSF gene transduction, as discussed in Section 2. The short-term culture technique developed for this study does not employ any drug selectable marker and thus maintains the potential representation of antigenic diversity while expanding cell numbers up to doses not previously studied in humans. Second, non-transduced RCC tumor cells in these short-term culture conditions also secrete low levels of GM-CSF as discussed in Section 2--albeit far below those levels set for GM-CSF gene transduced cells in this trial. Contribution of GM-CSF gene transfer itself to toxicities that follow can best be studied by this separation of cell dose from cell dose plus high-level GM-CSF secretion. Third, although the patient groups are small and are not intended to be equalized for many variables (see Biostatistics, Section 5), large differences in biological outcome between the two treatments in a pilot study could shape phase II trial design if equal patient safety is established.

Lastly, this human trial is intentionally designed to test the relevance of the murine gene therapy tumor models that engendered it. If predictions created by these models are confirmed in this trial, specifically that the local expression of GM-CSF by gene transduced, irradiated tumor vaccines induce human tumor specific immune responses more readily than non-transduced vaccines, then this experimental research direction can be welcomed as relevant to clinical cancer therapy.

3.2 THREE STAGE PHASE I TRIAL

This trial will be conducted in three stages. In the surgical stage (Stage 1), nephrectomy will be followed by vaccine preparation. In Stage 2 of the trial, eligible patients following surgery will be treated with cell therapy and evaluated for acute toxicities and induction of immune responses in the short-term. In Stage 3 of the trial, patients will be evaluated over the long-term (including autopsy) for late onset toxicities and correlation of immune response assays with clinical course. If patients show progression of their disease in Stage 2 or 3 of the trial, they may elect IL-2 therapy if they have not previously received it, treatment with other investigational agents, or best supportive care provided at the Johns Hopkins Oncology Center. In all cases, however, patients will continue scheduled follow-up for the observational stage of this trial.

3.3 STAGE 1: SURGERY, RECOVERY, AND CELL PREPARATION

Stage 1 of the trial will involve clinical and laboratory evaluation, and determination that candidate Stage III T 4b and Stage IV RCC patients are safe surgical candidates. Pathologic diagnosis and staging of disease will be completed during surgery (Figure 7). Surgical and medical eligibility criteria are delineated in Section 4.0. Prior to final enrollment, patients will sign informed consent in accordance with the institutional procedures for clinical investigation at the Johns Hopkins Oncology Center.

Provided the diagnosis of RCC is histologically confirmed at resection, one day after nephrectomy, patients will be randomized to cell preparation either with non-transduced tumor vaccine cells or with tumor vaccines transduced with the GM-CSF gene. Autologous vaccine will be prepared accordingly at Somatix Therapy Corporation for patients during their recovery from surgery. Blinded randomization will occur in the Biostatistics Office of the Johns Hopkins Oncology Center by the methods described in Section 5. Neither patients nor doctors will know prior to surgery which treatment arm will be assigned. Randomization is designed only to assign patients impartially to either treatment arm--not to equalize for all patient characteristics. After randomization, patients and their physicians will be blinded until conclusion of Stage 2 is completed for the individual patient. At the completion of stage 2, both patient and clinical investigator will be informed of assigned cell preparation for the individual patient.

During the post operative recovery period, vaccine cells plus or minus GM-CSF gene transfer will be expanded and ultimately lethally irradiated as described in Appendix 14.C. If the post-operative recovery is uncomplicated, each patient will be completely re-evaluated seven days before planned vaccination (day -7). If still eligible by all criteria in Section 4.0, the patients will receive pretreatment staging CT scans of metastatic disease sites and baseline immune function testing as described in Section 7.

If patients develop post-operative complications which require ongoing medical management that precludes experimental vaccine therapy, they will be

FIGURE 7 STUDY TIME COURSE

BASIC INVESTIGATION

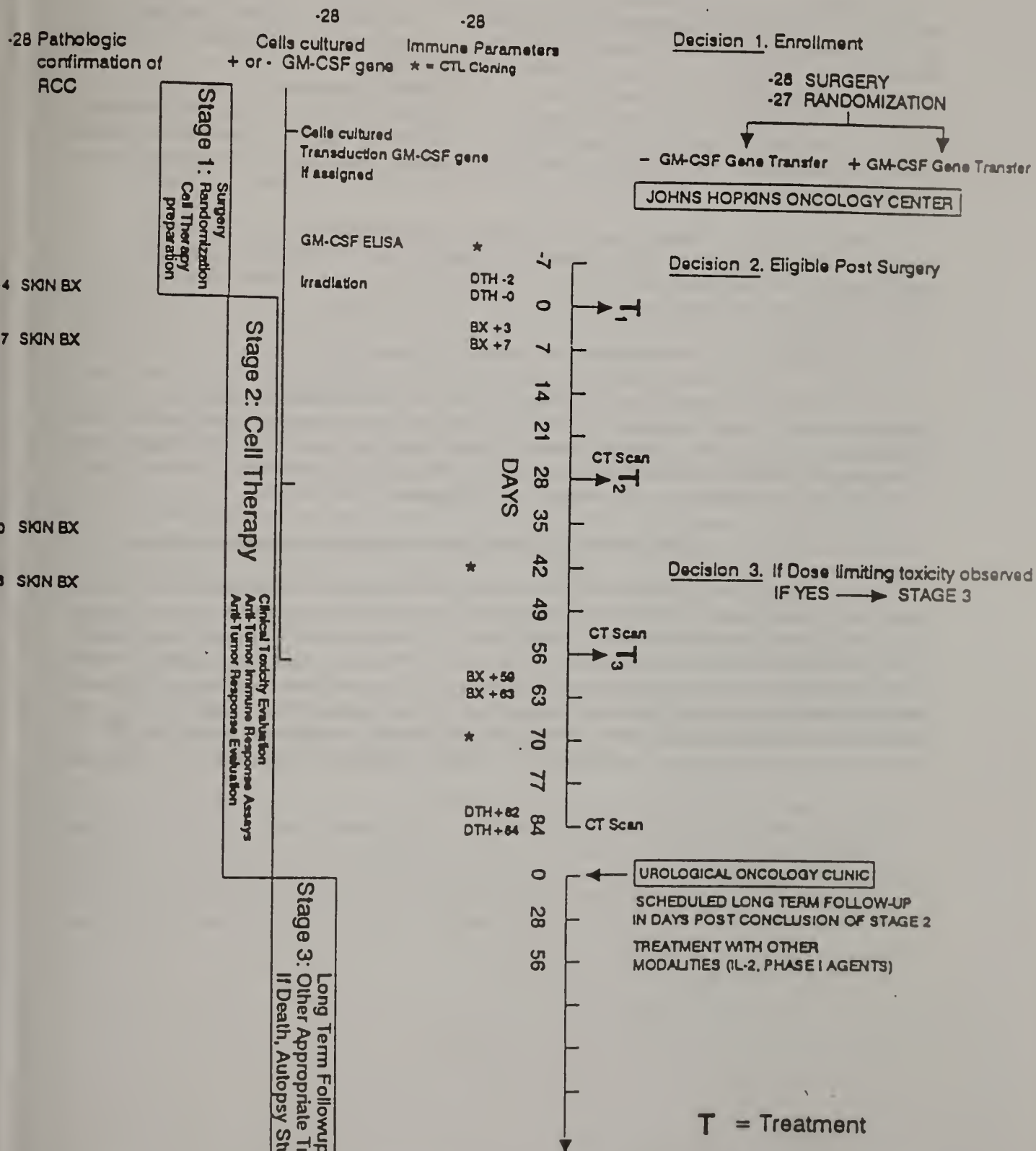
CLINICAL INVESTIGATION

PATHOLOGY

CELL THERAPY
PREPARATION

IMMUNE RESPONSE
STUDIES

DEPARTMENT OF UROLOGY
PRE-OPERATIVE EVALUATION



If patients develop post-operative complications which require ongoing medical management that precludes experimental vaccine therapy, they will be assigned directly to Stage 3 of the trial. Post-operative complications excluding eligibility for vaccination are described in Section 4.0.

Vaccination is scheduled to begin on Day 0 of Stage 2 of the trial, generally 28 days post-nephrectomy. Up to, but no more, than 42 days will be allowed postoperatively if additional time is required to propagate the number of RCC cells needed for 3 full courses of treatment. If at day 35 postoperatively or at the upper limit allowed for 4 passages of cells in culture, it appears that insufficient cells will be available for all three vaccinations at the assigned Dose Level, the patient will be treated on day 42 post-nephrectomy at the highest possible specified Dose Level allowing 3 complete treatments. The overall protocol time table is shown in Figure 7.

Patients will not be given fractions of an assigned vaccine dose. Technical failures of vaccine preparation resulting in RCC cell numbers insufficient to complete vaccination courses at the lowest Dose Level will force assignment of the patient to Stage 3 of the trial.

3.4 STAGE 2: VACCINATION AND TOXICITY EVALUATION

In Stage 2 of the trial, patients will be vaccinated on day 0. Evaluation for local and systemic toxicities and induction of antitumor immune responses will follow. Also in both Stage 2 and in Stage 3 of the trial, any evidence of autoimmunity will be assessed. The scope, rationale, and timing of these observations are delineated in Sections 7, 8, and 9.

3.4.a. Starting Vaccine Cell Dose and Dose Escalation Scheme

Tumor cell dose will be escalated in 10-fold increments until Dose Level 4 -- the upper limit feasible guided by our pre-clinical studies (Table 12). Four Dose Levels are set. Dose Level 1 will be 4×10^6 cells, Dose Level 2 will be 4×10^7 cells, Dose Level 3 will be 4×10^8 cells, and Dose Level 4 will be 1×10^9 cells. No individual patient will be treated with escalating doses. The method of dose escalation is described in the legend for Table 12. Accrual and randomization will continue until every treatment position is evaluated on both arms of the study, or dose limiting toxicities are observed.

TABLE 12

DOSE ESCALATION SCHEME

PARALLEL DOSE ESCALATION STUDIES

RCC CELLS PREPARED WITHOUT
GENE TRANSDUCTIONRCC CELLS PREPARED WITH
GM-CSF GENE TRANSDUCTION

DOSE LEVEL 4	— — —	1×10^9	— — —	$1 \times 10^9 + \text{GM-CSF}$
DOSE LEVEL 3	— — —	4×10^8	— — —	$4 \times 10^8 + \text{GM-CSF}$
DOSE LEVEL 2	— — —	4×10^7	— — —	$4 \times 10^7 + \text{GM-CSF}$
DOSE LEVEL 1	— — —	4×10^6	— — —	$4 \times 10^6 + \text{GM-CSF}$

└───┐
RANDOMIZED TO CELL PREPARATION└───┘

DOSE ESCALATION SCHEME:

After randomization to cell preparation, patients will be assigned to a treatment position on one arm of the study. Randomization to either arm of the study will continue until every treatment position is evaluated, or dose limiting toxicities are observed. If one arm of the study completes accrual before the other, randomization will stop to expedite study completion. Each bar above represents a position to be filled. Starting with Dose Level 1, every patient treatment position must accrue and represent 1 completed, evaluable 28 day course at the Dose Level under study before dose escalation proceeds to the next Dose Level. If one case of dose limiting toxicity is observed in the original set of treatment positions at a given Dose Level, three additional patients will be treated at that Dose Level to assess whether a dose limiting toxicity occurs again. If no further cases of dose-limiting toxicity are observed in this additional set of 3 patients, dose escalation will continue to the next level. If two cases of dose-limiting toxicity are observed at a Dose Level under evaluation, 3 additional patients will be treated at the next lower Dose Level to confirm the MTD is approached. (Sections 3,5, and 9) In the case of Dose Level 1, 2 cases of dose-limiting toxicities in a total set of 5 individual patients will close that arm of the study to further accrual. Dose Level 4 represents the feasible limits of short-term culture of RCC vaccine cells. Unlike the rules for the lower 3 Dose Levels, if sufficient cells are available for only 1 or 2 complete treatments at Dose Level 4, patients will be treated at Dose Level 4 and not dropped to a lower Dose Level to complete 3 courses. If no dose-limiting toxicities are observed for Dose Level 4, after 3 patients complete at least 1 treatment cycle, 3 additional patients will be assigned to confirm no dose limiting toxicities exist at the limits of achievable cell dose. No individual patient will be treated with escalating doses. Cumulative toxicity will be studied, if possible, by up to a total of 3 courses spaced every 28 days. In the event there is evidence of a partial response during treatment in either arm of the study, if sufficient vaccine cells remain, and if there is no evidence of hazardous cumulative toxicity, patients can continue beyond the 3 scheduled 28 day injections until exhaustion of prepared cells. However, to maintain a symmetric study of the cumulative toxicity of three cycles of injections, such a patient's treatment position may be re-opened for accrual to evaluate 3 complete treatment cycles in another patient.

CONTINGENCIES: If in preparation of vaccine cells, insufficient cells are available to complete 3 courses of treatment at the assigned Dose Level, the patient will be treated at the highest Dose Level allowing 3 complete courses. In this case, the treatment position will remain open. No dose fractions will be given. If Dose Level is dropped in this situation, the treatment position originally assigned will remain open for accrual, however. If after gene transfer GM-CSF secretion is below the lower limit of 24 ng/10⁶ cells/24 hr, the patient will be treated at the assigned Dose Level and be assessed for clinical toxicity; however, the original treatment position assigned will remain open for accrual to treatment with vaccine plus GM-CSF gene transfer.

3.4.b. GM-CSF Gene Expression in Vaccine Cells

Prior to cell therapy, tumor vaccine cells prepared for patients assigned to the non-transduced arm of the study will be assayed for native GM-CSF secretion by ELISA and data recorded. In the GM-CSF gene transduced arm of the trial, secretion of GM-CSF will be determined by ELISA prior to first injection and set at 24-48 ng/10⁶ vaccine cells/24 hr by the methods described in Appendix 14.C.

3.4.c. Schedule of Vaccine Administration

Cell therapy will be administered every 28 days for up to 3 complete courses. Spacing and location of vaccination sites are detailed in Table 13. The first dose for each patient will be administered as an inpatient, followed by 24-hour observation before discharge. If no immediate toxicities are observed, the subsequent two vaccinations will be given in the Johns Hopkins Oncology Center Outpatient Department with 8-hours of clinical observation prior to release for scheduled follow-up visits.

3.4.d. Vaccine Preparation and Formulation

Vaccine preparation, formulation, and quality assurance protocols are delineated in Appendix 14.C. Vaccines will be finally formulated in the Johns Hopkins Oncology Center Pharmacy and placed in 10 ml graduated syringes. Vaccine will be administered using a 23 gauge needle as described in Table 13.

TABLE 13		INJECTION TECHNIQUE			
DOSE LEVEL	DOSE INJECTED	CELLS/ml	INJECTION VOLUME	INJECTIONS	
4	1X10 ⁹	5X10 ⁷	0.5cc/site	4 (2id/2sq)	
		7.5X10 ⁷	1.0cc/site	12 (6id/6sq)	
3	4X10 ⁸	5X10 ⁷	0.5cc/site	16 (8id/8sq)	
2	4x10 ⁷	5x10 ⁷	0.2cc/site	4 (2id/2sq)	
1	4X10 ⁶	5X10 ⁶	0.2cc/site	4 (2id/2sq)	

1
Injections with a 23 gauge needle will be given in the patients' forearms and thighs following a grid pattern prescribed by a customized foam rubber template. On the grid each injection is 3 cm at needle entry from nearest neighbor injection. 50% of total injections will be intradermal; 50% subcutaneous--alternating intradermal/subcutaneous in the cephalad-caudad axis. Cycle 1 vaccination limb will be right anterior thigh, Cycle 2 vaccination limb will be non-dominant arm anterior fore-arm, Cycle 3 vaccination limb will be left anterior thigh.

3.5 STAGE 3: LONG-TERM FOLLOW-UP

Protocol patients will maintain scheduled long-term follow-up at the Johns Hopkins Oncology Center. If their disease progresses at any time during the trial, patients will be offered treatment with other therapies including IL-2 or phase I drugs under study if that is medically appropriate. They may also choose to remain on the study. However, they will be asked to continue scheduled follow-up at the Johns Hopkins Oncology for continuity of care and as a part of long-term follow up for this study. The timing and nature of routine studies including assessment of any late onset auto-immune toxicity are described in Section 7. Presence and duration of patient tumor specific immune responses will be tested during scheduled Stage 3 clinic visits as described in Section 7 as well. Patients and families will have been asked for prior authorization for autopsy in the event of death, as described in Appendix 14.H. Autopsies will be performed at the Johns Hopkins Hospital under the direction of the study's pathologist, Dr. Audrey Lazenby. The strategy of the detailed clinical-pathologic analysis to be followed is described in Section 9.

3.6 RATIONALE FOR STUDY DESIGN AND PREDICTED TOXICITIES

3.6.a. Surgery

Surgery is essential for procurement of RCC tumor cells for therapeutic presentation of tumor-specific antigens in the autologous vaccine after short-term culture. Nephrectomy in stage III T4b and stage IV disease has become an essential component of RCC experimental immunotherapy strategies as reviewed in Section 2. Nephrectomy is also performed in this setting to forestall major morbidity which can attend the increasing growth of the primary tumor. The intra-operative mortality rate at Johns Hopkins for radical nephrectomy in RCC patients pre-selected by this study's eligibility criteria is under 2%.

3.6.b. Randomization

Randomization to treatment arm is blinded to both patients and clinical investigators in order to assign treatment impartially to plus or minus gene transduction one day after nephrectomy (Section 5 describes methodology). Randomization is not intended in this pilot study as a tool to eliminate all bias or to justify a type I error rate (alpha level) of statistical hypotheses tests. In addition, the blinded clinical investigators will be assisted in more objective evaluation of subjective, non-quantitative assessments of toxicity (minor symptoms) without biases about the type of cell preparation during Stage 2 of the trial.

3.6.c. Starting Vaccine Tumor Cell Number and Treatment Cycles

The starting Dose Level 4×10^6 RCC vaccine cells is chosen for its apparent safety as it represents 1/200th the maximum reported dose of tumor vaccine cells without propagation in short-term culture administered in previous reported trials.⁵⁶⁻⁵⁹ A direct comparison between this trial and previous trials is limited: no study has prepared RCC vaccine cells in primary culture like this one. Despite local, reversible, cutaneous reactions of erythema and ulceration of 1-2 weeks duration following vaccination, life threatening toxicities were not described in earlier human tumor vaccine studies.⁵⁶⁻⁵⁹ The ulcerations were thought due to adjuvants such as BCG used with uncultured tumor cells. The choice of 3 treatments is arbitrary to evaluate cumulative toxicity. In our preclinical models multiple doses have not shown increased efficacy over a single dose.

3.6.d. Highest Dose Level Choice

Dose Level 4, at 1×10^9 vaccine cells is chosen as an upper limit because experience has shown that this limit can be reached within 4 tissue culture passages on occasions when one starts with large viable tumors. In order to preserve maximum antigenicity of tumor cells in primary culture, candidate vaccine cells will only be expanded to a maximum of 4 passages prior to use. Immunogenicity has been shown to decrease with long-term culture of cancer cell lines although precise data for humans on this point are not available.

3.6.e. Choice of GM-CSF Dose

The strategy of GM-CSF dosing to be followed in this study is set to enhance the evaluation of the paracrine effects of the cytokine on the immune response as opposed to the systemic effects of GM-CSF on hematopoiesis. Our preclinical studies suggest that a range of GM-CSF secretion of 24 to 48 ng/ 10^6 cells/24 hours will achieve this result and insure maximal specific antitumor immunity against later challenge by the inoculation of wild-type tumor cells.

In this study we are defining the amount of immunostimulating cytokine secreted by a fixed number of autologous tumor antigen expressed in ng/vaccine cells/per 24 hours. This form of dosing is different from the actual total number of retrovirally inserted genes (integrants) per vaccine tumor cell. In other words, the total numbers of GM-CSF genes inserted per vaccine cell could be different between vaccine preparations, but the absolute amount of secretion of the GM-CSF gene product into the microenvironment per hour is measurable, and can be standardized to the desired secretion rate of 24-48 ng/ 10^6 cells/24 hr called for in this study. Final cell injection formulation is described in Appendix 14.C.

The effect of fixing the range of secretion of GM-CSF from 24-48 ng/ 10^6 cells/24 hours on the daily dose of the cytokine received by the patients may be seen in Table 14. The total daily dose received by patients will increase

in relation to the total number of RCC tumor cells inoculated (the Dose Level of the vaccine). A wide range of daily doses will be received by the patients. Only at Dose Level 4 will the daily dose of GM-CSF approach amounts which have been shown to stimulate hematopoiesis in patients (Table 14).

TABLE 14 MAXIMUM POTENTIAL DAILY SYSTEMIC EXPOSURE TO GM-CSF
POST CELL INJECTION

DOSE LEVEL	TREATMENT CELL DOSE	MAXIMUM DAILY GM-CSF EXPOSURE (CELL DOSE x 48ng/10 ⁶ /DAY)
4	1 x 10 ⁹	48,000 ng (48 mcg)
3	4 x 10 ⁸	19,200 ng (19.2 mcg)
2	4 x 10 ⁷	1,920 ng (1.92 mcg)
1	4 x 10 ⁶	192 ng (.192 mcg)

The maximum, daily, systemic exposure to GM-CSF protein (assuming 100% bio-availability) in this study per day at maximum dose (Dose Level 4) appears safe. It is significantly less than (about 11%) the published of GM-CSF MTD by patients given the cytokine, either IV or subcutaneously. The MTD (defined as the occurrence of reversible grade 3-4 toxicity in at least 2/3rd of patients was determined to be 10 mcg/kg/day subcutaneously or 700 mcg daily.^{59,60,61} As a reference point, 1-6 mcg/kg/day is an approved usual starting dose in oncology patients using IV rhu GM-CSF, or 70 to 420 mcg per day. The systemic pharmacokinetics of GM-CSF secreted by B16 melanoma tumor vaccine are presented in Appendix 14.A.1, Figure 1. Nevertheless, unforeseen and synergistic toxicities between irradiated tumor cells and high level paracrine GM-CSF in the subcutaneous space may exist. If so, the two arm study design should enhance the likelihood of their detection.

3.6.f. Choice of Both Intradermal and Subcutaneous Vaccination Routes

The decision to do both intradermal and subcutaneous injections comes from our inability to use our murine models to determine which route is preferable. Mice do not have an intradermal space large enough to accommodate large cell doses. This issue may require head to head comparison in later phase II clinical studies provided the safety of each route of administration is shown in this trial.

There is evidence that the intradermal inoculation site could be superior in humans. In leprosy, for example, GM-CSF given intradermally has more efficacy in clearing infections than given subcutaneously. Intradermal vaccination appears superior to subcutaneous vaccination in immunization with Hepatitis B vaccine as well.^{62,63,64,65} For these reasons, the intradermal route was not eliminated for clinical evaluation in this study in favor of the

clinically more convenient subcutaneous route.

Injections will be alternated in a grid pattern. Equivalent numbers of injections of subcutaneous and intradermal doses in the same limb should allow local toxicities between the two routes to be compared by parameters described in Section 7 using the patient as his or her own control. India Ink or tatoos might confound the dermatologic evaluation following vaccination; for this trial a custom-made, foam rubber grid will be made for each treated limb to ensure injection positioning and evaluation of vaccination sites over time.

3.6.g. Potential Toxicities

Potential Toxicity from GM-CSF Secretion

Toxicities from GM-CSF gene transduced human RCC tumor vaccines are difficult to predict. In over 40 separate experiments (>400 mice) escalating the cell dosage of B16 melanoma tumor vaccine or RENCA murine RCC with fixed secretion of GM-CSF has shown only reversible lymphadenopathy and reversible subcutaneous swelling. (Appendix 14.A.2) No ulcerations in the vaccinated limb were observed. Common clinical toxicities predicted from previous phase I and phase II studies of recombinant GM-CSF given by subcutaneous injection are fever, malaise, and bone pain putatively from proliferation of bone marrow precursors. Toxicities from leukocytosis may also be encountered. In the human pharmacokinetic study of single dose subcutaneous GM-CSF cited above, a 700 mcg subcutaneous injection achieved a serum level of >1ng/mL, which corresponds to the concentration of rhuGM-CSF supporting near-maximum proliferation of human myeloid progenitors in vitro. Less common and case reports of toxicities for GM-CSF are listed below and referenced in Table 15.

TABLE 15 POTENTIAL TOXICITIES OF GM-CSF SECRETION

<u>Systemic Toxicity from GM-CSF Administration</u>	<u>Reference</u>
Fever	1
Transient Leucopenia	2
Transient Leukocytosis	3
Diarrhea	4
Weight Gain/Fluid Retention	4,5
Malaise	5,6
Bone Pain	5,6
Arthralgia	5,6
Liver Transaminase Elevation	6
Hemolysis	1,7
Rheumatoid Arthritis	5,6
Leukoclastic Vasculitis	8
Renal Failure (after bone marrow transplantation)	5,9
Severe Hypocalcaemia with Leukocytosis	10
Eosinophilia, Splenic Hematopoiesis	11,15

TABLE 15 CONTINUED

Pericarditis/Pleural Effusions/Capillary Leak at 60ng/kg	14
Pulmonary Embolism	14
Hypoalbuminemia	16
Reversible Thyroiditis	17
<u>Local Toxicities from GM-CSF Administration</u>	
Pustular Eruption of Injection Site	11
Necrotizing Vasculitis (Hemorrhagic Bullae at Injection Site)	12
Erythema, pruritus at injection Site	12
Recall Erythema at previous injection Site	12
General papular rash	12
Phlebitis at injection site	13, 14, 18

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Potential Autoimmune Toxicities

The potential of this form of therapy to induce late onset auto-immune disease makes Stage 3 of the trial essential in the study design--namely long-term follow up at the Johns Hopkins Oncology Center. Measurements and timing of clinical indicators of auto-immunity are delineated in Section 7.

Using the RENCA murine RCC tumor model transduced with murine GM-CSF, we could detect no histologic evidence of induced glomerulonephritis or auto-immune tubular damage to the normal kidneys. Renal function did not deteriorate by serial creatinine determinations in these mice either (Appendix

14.A.2). Nevertheless, as these patients will be uninephric, special attention will focus on detecting early evidence of renal deterioration. A uninephric baseline DPG renal scan (as the most sensitive test of GFR currently available clinically) will be obtained before day 0 of Stage 2 in order to follow and quantitate the degree of renal injury induced if it does occur.

Potential Toxicity from MFG Retroviral Vector

The generation of genetically modified tumor cells capable of secreting the desired quantity of human GM-CSF will be accomplished by retroviral mediated gene transfer, a highly efficient means of transferring genes into mammalian cells. This method involves the use of genetically engineered viruses which are capable of entering cells and inserting their genetic material into host cell chromosomal DNA at high efficiency, yet unlike normal replication competent retroviruses, are not capable of producing any progeny virus after transduction of the target cell.

Previous clinical protocols which have involved the use of such replication-deficient recombinant viruses have not revealed any toxicities associated with the gene transfer process. However, several areas of concern about the gene transfer process have been theorized and are worthy of mention. A first concern relates to the possibility that the recombinant viral stocks used for transduction of the tumor cells may contain low levels of replication-competent virus. In animal models, the chronic infection of host cells by certain retroviruses has been shown to contribute to the development of neoplastic disease, by virtue of the capacity of specific insertions of proviral DNA into chromosomal DNA to alter the expression of growth control and other genes. Accordingly, the presence of replication competent virus in the virus stocks used to transduce tumor cells could lead to the chronic propagation of replication competent virus in vivo, after transplantation of the transduced cells, and potentially contribute to the development of disease in the patient. The presence of replication competent virus in vivo would also make possible the transfer of the GM-CSF gene to other cells. Finally, even in the absence of replication competent virus, the transmission of the GM-CSF sequences to either tumor cells or contaminating normal cells could affect the growth properties of those cells in an undesirable way.

While aware of these issues, we believe they pose no significant risk in this particular clinical protocol. First, the system for generating recombinant virus that we will employ has been designed to eliminate the possibility of helper virus generation, and we have developed extremely sensitive assays for monitoring the purity of our virus stocks that can detect the presence of a single virus particle in the stocks, should it exist. To date, we have no evidence of helper virus production. It is also worth noting that the packaging cell system we will employ possesses additional features from those engineered in the packaging cell system that has been used most frequently in the past for the generation of clinical grade viruses (Appendix 14.B).

Issues relating to the potential risks associated with proviral insertion itself are more difficult to evaluate, since no in vitro assays have been able to detect such events. In contrast to previous clinical protocols involving live, transduced tumor cells, however, the protocol described here is different in that the use of irradiated cells as vaccines preclude the possibility that the growth properties of the vaccine cells might be altered by the gene transfer process. We believe that the use of irradiated cells is, therefore, extremely important from patient safety standpoint.

4.0 PATIENT ELIGIBILITY

4.1 Prior to final enrollment in the study, patients must have a documented diagnosis of Stage III T4b or Stage IV RCC after surgery. There must be unambiguous histologic confirmation that the primary tumor is renal cell carcinoma. If a diagnosis of RCC has already been established by needle biopsy, it must be reconfirmed histologically at nephrectomy.

4.2 Patients must not be pregnant and must be greater than 18 years of age. There must be no second form of cancer present at enrollment.

4.3 Patients must have a performance status (Eastern Cooperative Oncology Group of 0 or 1 and must have an estimated life expectancy of 6 months or more.

4.4 Patients must sign standard informed consent for surgery and general anesthesia at the Johns Hopkins Hospital. In addition, patients must give written informed consent to participate in this trial (Section 12) prior to surgery.

4.5 Patients must have had no other recent major surgery, i.e., be at least 1 month post any surgical procedure. In addition, no immunosuppressive therapy including topical or oral corticosteroids, radiotherapy, or cytotoxic therapy can have been administered within 1 month prior to enrollment. Patients must be at least one month post any form of immunotherapy (for example, IL-2, interferon-alpha, ALT therapy) prior to enrollment.

4.6 Patients must have adequate bone marrow function ($WBC \geq 4000/uL$, platelets $\geq 100,000/uL$), hepatic function (total bilirubin ≤ 1.5 mg/d:) and at day -7 to Stage 2 of the trial must have adequate uninephric renal function (creatinine ≤ 2.0 mg/dL). Patients must be seronegative for HIV, also. Patients must have no documented history of the following auto-immune diseases: systemic lupus erythematosus, sarcoidosis, rheumatoid arthritis, autoimmune hemolytic anemia, auto-immune thyroiditis, glomerulonephritis, or vasculitis.

4.7 Patients must be negative for brain metastases by enhanced cranial CT or MRI prior to final enrollment.

4.8 No active infections requiring treatment may be present, including

surgical site infections following nephrectomy prior to final eligibility on day -7 for Stage 2 of the trial. Postoperative complications, including deep venous thrombosis or pulmonary embolism, will exclude eligibility for Stage 2 and result in assignment to Stage 3 of the trial. Any other postoperative medical problem of sufficient severity to require ongoing medical management and make experimental therapy unduly hazardous will be grounds for exclusion of Stage 2 (decision on day-7) and assignment to Stage 3 of the trial.

4.9 Prior to enrollment, investigators must inform both patients and family members that in the event of a death on the study at anytime, permission will be sought for autopsy. Patient or family refusal or indecision is in no way an exclusion criteria for enrollment.

4.10 Patient must have no other pre-existing medical problems of sufficient severity to limit full compliance with the study or expose them to undue risk.

5.0 BIOSTATISTICS

5.1 Study Design

The design of this study is somewhat unusual for a Phase I dose escalation. In particular, we intend to randomly assign patients to one of two treatment groups: one with GM-CSF gene transduction of cells and one without. Randomization is frequently used both to reduce bias and to justify the type I error rate (alpha level) of statistical hypothesis tests. In this study, the purpose of randomization is to insure that patients are assigned to the respective treatment groups impartially with regard to "gene therapy"--not to justify statistical hypothesis tests.

5.2 Sample Size and Accrual

This trial employs a simple Phase I dose escalation scheme. The lowest two doses (4×10^6 and 4×10^7 cells) will accrue only two patients in each group. This is because we do not expect serious toxicities at these Dose Levels. The remaining two groups will employ three patients per treatment group. In addition, in the gene transfer group at the highest dose (1×10^9 cells), three additional patients will be accrued to obtain additional experience. In the event that 2 dose limiting toxicities are observed at any dose, three additional patients will be accrued at the next lower dose as described in Section 3. The rate of accrual on this study is limited by the capability to prepare multiple vaccines simultaneously. We expect to be able to treat 6-8 patients per month. Thus, Stage 2 of the trial will last for approximately 6 to 8 months depending on toxicities observed.

5.3 Randomization

The study will employ equal randomization between the two treatment groups. All matters concerning registration, randomization, and treatment assignment will be conducted through the Oncology Biostatistics Office in the

Johns Hopkins Oncology Center. One day after nephrectomy, if the patient has histologically confirmed renal cell carcinoma, investigators will call a centralized registration/ randomization desk in Oncology Biostatistics. A brief eligibility check will be obtained before patients are randomly assigned to a treatment group. Randomization will be blocked in such a way that accrual for a lowest Dose Level will be filled prior to assigning any patients to the next higher Dose Level. If there are no dose-limiting toxicities after accrual of all patients required on the randomized portion of the study, 3 additional patients will be assigned non-randomly in the gene transduced group at Dose Level 4.

5.4 Early Stopping

This study will employ the usual guidelines for Phase I dose escalation designs. In particular, dose-limiting toxicities observed at one Dose Level will require that patients be treated at the previous Dose Level in order to find the MTD. Because of the strict dose escalation design, we will employ no formal biostatistical guidelines for early termination of the study.

5.5 Analysis of Key Endpoints

The primary statistical endpoint of this study is to estimate the MTD if it exists in the dose range to be studied. The criteria for defining the MTD are outlined in sections 3 and 9. The assessment of this endpoint will follow the theory and practice recommended by Storer (1). Although recent theory and simulations have suggested that alternative phase I design may be more efficient (2-5), the present study employs a traditional phase I dose escalation design and analysis because the ethical criteria for alternative designs cannot be met.

A second goal of this study is to determine the feasibility of this new form of treatment. The gene transfer success rate will be determined by the success rate of establishing short term cultures that provide the assigned dose level of RCC cells for three doses after full expansion. A second endpoint of feasibility will be the success rate of MFG vector GM-CSF gene transduction during vaccine preparation in yielding a secretion rate of 24-48ng/10⁶ cells per 24 hour versus total attempts. These success rates will be determined as a proportion in the usual fashion where the denominator includes all patients who meet the eligibility criteria. Exact binomial confidence limits will be placed on each estimated proportion.

A third goal of this study is to estimate the frequency of toxicities due to therapy, particular renal injury. These will be characterized according to the National Cancer Institute Common Toxicity Criteria and exact binomial confidence limits will be used to characterize the precision of the estimate. Other toxicities of interest include local and systemic toxicities associated with the RCC cells and autoimmunity. These will be treated in a similar fashion. Large differences in toxicity between the randomized groups will be characterized and noted.

The remaining outcome parameters of this study are more descriptive in nature. It is not possible to describe fully an analytic plan for these outcomes. The approach to the analysis must be interactive and will depend upon preliminary and descriptive results. Nevertheless, the following section provides a general introduction to the approach that will be used. Study parameters are listed in section 7.3 of the protocol. Variables measured on a continuous scale, such as laboratory measurements, will be characterized by summary statistics (mean and standard deviation). The randomized study design permits a valid comparison of means between the treatment groups. However, because of the small sample size, we will not emphasize such comparisons. If they are made, standard tests such as t-tests or their non-parametric analogs will be performed.

Other variables are dichotomous or categorical in outcome such as the results of symptom inventory, presence or absence of auto antibodies, and response to allogenic stimulator cells. These variables will be summarized using counts and proportions with exact binomial confidence limits. Here again, any comparisons between the treatment groups will be made using either Fisher's exact test or its multi-dimensional analog.

Pharmacokinetic parameters will be estimated, when possible, using standard compartmental models. Parameter estimates and normal theory standard errors will be calculated by fitting using weighted least squares. The relationship between pharmacokinetic parameters and clinical outcomes such as toxicity will be assessed using linear regression or a related technique.

We do not anticipate the need for detailed multivariate analysis. Also, the sample size employed will not support the precise estimate of covariate effects adjusted for other prognostic factors.

Biostatistics References

1. Storer, B.E. Design and Analysis of Phase I Clinical Trials. Biometrics, 45:925-937, 1989.
2. O'Quigley, J. Estimating the Probability of Toxicity at the Recommended Dose Following a Phase I Clinical Trial in Cancer. Biometrics, 48:853-862, 1992.
3. O'Quigley, kJ., and Chevret, S. Methods for Dose Finding Studies in Cancer Clinical Trials: A Review in Results of a Monte Carlo Study. Statistics in Medicine, 10: 1647-1664, 1991.
4. O'Quigley, J., Pepe, M. and Fisher, L. Continual Reassessment Method: A Practical Design for Phase I Clinical Trials in Cancer. Biometrics, 46:33-48, 1990.
5. Gatsonis, C. and Greenhouse, J.B. Bayesian Methods for Phase I Clinical Trials. Statistics in Medicine, 11:1377-1389, 1992.

6.0 REGISTRATION OF PATIENTS

All patients eligible for this phase I study will be discussed and evaluated by the principal investigator and co-investigators before entry into the study. Alternatives to participation, including treatment with approved systemic IL-2, or phase I agents will be considered. The on-study requirements for clinical laboratory work, baseline immunological studies, and clinical evaluation are to be completed and entered into Biostatistics Office trial computerized data base 5 days before Day 0 of Stage 2 of the trial.

7.0 STUDY PARAMETERS

7.1 RATIONALE FOR CLINICAL TOXICITY EVALUATION

This treatment is not a cytotoxic drug. No dose limiting toxicities may be observed even at Dose Level 4. Nevertheless, the common toxicity criteria of the NCI and the cutaneous toxicity scale unique to this trial described in Appendix 14.E and Appendix 14.F will be employed. After treatment, patients will be seen as outpatients on a frequent scheduled basis. After 3 cycles of treatment are ended (day 84), patients will be followed in Stage 3 of the study, and return for scheduled evaluations or sooner as clinically indicated.

7.2 RATIONALE FOR IMMUNOLOGIC STUDIES

As described, the concepts being tested in this study were derived from murine model systems that enabled screening of a number tumor/cytokine combinations for efficacy. This allows for initial dissection of the underlying immunological mechanisms responsible for the observed anti-tumor effects. One objective of the current study is to determine the validity of the mouse model systems as predictors of anti-tumor immunological activity in humans.

The "gold-standard" measure of the anti-tumor immune response in the murine model is protection against or cure of a defined live tumor challenge. If in stage IV renal cell tumor patients, metastatic regression following treatment is not observed, this may not mean that the cell therapy is inactive, rather, that the "readout" at high tumor burdens is too insensitive to show the presence of an active anti-tumor immune response. In fact, it is likely this may be the case in this phase I study using patients with advanced RCC, and that significant immunologic activity could be missed if tumor response were the sole criterion of potential therapeutic activity.

Consequently, our preclinical studies have focused on a variety of measurements that characterize the specific features of the antitumor immune response to GM-CSF transduced tumor vaccines. These enable an attempt at correlation with overall protection against tumor challenge, as well as giving some insight into the immunologic mechanism underlying the observed effect. Accordingly, these measurements will be made in patients treated in the current study.

7.2.a. BIOPSY OF THE VACCINE SITE. Preclinical data show this to be one of the most sensitive measurements of the effect of local tumor production of GM-CSF. Histologically, the response to subcutaneously injected irradiated non-transduced tumor varies, depending on the "immunogenicity" of the tumor. The histopathologic response is unmistakably different when the tumor is transduced to produce GM-CSF, with the former showing a surrounding cuff of lymphocytes, while the latter has a profound infiltration of monocytes, macrophages, eosinophils, and later, lymphocytes and PMNs. The kinetics of this response in the mouse are that by 5 to 7 days there is little viable tumor left. It is acknowledged that at Dose Levels 1 and 2, where the dose is divided into four injections per cycle, the biopsy of the vaccine site (once on day three and again on day seven) is in a sense "removing" a modest fraction of the therapeutic agent. This is felt to be justifiable on several accounts. Firstly, the punch biopsies are far from excisional, leaving behind a sizeable component of the vaccination at that site. Secondly, if the kinetics of the human response mirror that seen in the mouse, by the time of the day seven biopsy, the critical events of the antigen processing and presentation may have already occurred. Finally, in all Dose Levels, the majority of the vaccine is left undisturbed at the other sites for each cycle, and the amount removed is relatively constant between patient groups.

7.2.b. DTH RESPONSE. The delayed type hypersensitivity response is a well characterized in vivo measurement of antigen specific T cell mediated responses. McCune and colleagues reported a correlation between the development of a DTH response to autologous tumor and clinical response in patients with advanced renal cell carcinoma.³⁷ In addition to the standard measurements of induration and erythema, we propose to biopsy the DTH site. Our preclinical data shows that the histologic appearance of the response to the "priming" GM-CSF producing vaccine is dramatically different than that of the contralateral non-GM-CSF producing "challenge" site. This qualitative difference between the histology of the vaccine site and the challenge site was also noted in other tumor/cytokine combinations. Some vaccines (such as B16 melanoma/IL-2) showed impressive infiltration of the vaccine site, but very little infiltrate at the site of the tumor challenge. The response to the non-cytokine producing tumor challenge apparently more closely reflects the nature of the systemic immune response which has the greatest relevance to activity at sites of distant metastases. Ideally, it would be helpful to have biopsies of actual metastases, but in this patient population this could incur the risk of serious morbidity. The biopsy of the DTH response (i.e. irradiated non-transduced autologous tumor injected subcutaneously) may provide similar information with substantially less risk to the patient.

Given the possibility that non-transduced irradiated autologous tumor may be significantly immunogenic, it must be acknowledged that the DTH testing may alter that which it is intended to measure. In fact, for patients randomized to no GM-CSF transduction at Dose Level one, the "pretreatment DTH" testing amounts to 25% of the dose of the first cycle. While this dose may have some priming effect, it is the change in the DTH response that occurs with subsequent vaccination that will be the critical determinant of immunological activity. Also, our preclinical data suggest that total cell

dose is an important parameter in the magnitude of the immune response, such that at higher Dose Levels, the contribution of 1×10^6 irradiated tumor cells used in DTH testing may be insignificant to the immunizing dose of 10^8 or 10^9 cells.

7.2.c. DRAINING LYMPH NODE ANALYSIS - A consistent feature of our preclinical model is substantial enlargement of the draining lymph nodes of the vaccine site by day seven after vaccination with GM-CSF transduced tumor vaccine. (Appendix 14.A.4) The amount of enlargement is substantially greater than when irradiated non-transduced tumor is used alone. Histologic analysis shows both paracortical hypertrophy (T cell dependent zone) as well as some increase in the germinal center regions (B cell areas). Functional studies demonstrate a profound increase in the proliferation of $CD4^+$ T cells relative to that of unprimed lymph node cells, or those primed with non-transduced irradiated tumor alone. Analysis of the draining lymph node cells for endogenous cytokine mRNA suggests the initiation of a cascade of cytokine production including IL4 and IL6. Finally, by day 7, tumor specific $CD8^+$ CTL may be derived after in vitro expansion from draining lymph node cells. These observations delineate the early events that characterize the initial "priming" events after tumor cell/GM-CSF vaccination in murine models. This constellation of parameters will be examined in this study as an indication of immunological activation, as well as to potentially derive reagents such as cytotoxic T-cell clones that may aid in defining the tumor specific peptides that are the target of immunologic recognition. For these reasons, if the right draining inguinal nodes increase two fold or greater during the first treatment cycle lymph node biopsy will be performed in this study and assayed as described in the next section.

7.3 STUDY OBSERVATIONS AND FREQUENCY

PARAMETER

FREQUENCY OF EVALUATION

I. CLINICAL TOXICITY

History and physical examination	D
Vital Signs and Weight	A
Performance status	A
HCT, WBC, platelet counts	B
Differential, absolute eosinophil count	B
Electrolytes	B
BUN, creatinine	B
Total protein/albumin	C
Calcium/phosphate	C
Alkaline phosphatase	C
Total direct bilirubin	C
SGOT, SGPT	C
Uric acid	C
Glucose	B
ESR	K
Urinalysis	B
Urine Micro-albumin Assay	B
Chest x-ray	M
EKG	M
Baseline DPG Renal Scan	F
Dermatologic Photography at Vaccine Site	A
Brief Symptom Inventory (Psychological Questionnaire)	O

II. GM-CSF PHARMACOKINETICS

Serum GM-CSF Levels	L
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III. AUTO-IMMUNE TOXICITY

Anti-nuclear antibody	D
Rheumatoid Factor	D
Thyroid Function Tests(TSH, T3,T4)	D
Anti-thyroid Antibodies	D

IV. IMMUNE RESPONSE PARAMETERS

FREQUENCY OF EVALUATIONPRE-TREATMENT

HLA typing	F
Anti-CD3 reactivity	L
Response panel of allogeneic stimulator cells	L
Peripheral Blood CTL to autologous tumor target	N

POST-TREATMENT

Injection site	
Punch biopsy/histopathology	H
Immunohistochemistry for effector cells	H

Draining lymph node biopsy

a) T-cell proliferation assay	I
b) CTL generation versus autologous tumor targets	I
c) Cell Surface Markers Flow Cytometry: IL-2 receptor, B-7	I
d) Histopathology	I

Delayed Type Hypersensitivity Reaction

a) Recall antigens Candida, Trichophyton, PPD	J
b) Irradiated autologous PBL control 1×10^6	J
c) Irradiated autologous tumor target cells 1×10^6 unpassaged from nephrectomy	J
d) Irradiated autologous normal renal cells 1×10^6 unpassaged from nephrectomy	J

V. EVALUATION OF ANTITUMOR RESPONSE/TUMOR PHENOTYPING

a) CT scan/MRI, chest, abdomen, pelvis	K
b) Serum markers of RCC tumor burden	
Urine beta-FGF	K
Serum Parathyroid Related Protein	K
Serum Ferritin	K
Plasma OA-519	K

KEY TO CODE OF FREQUENCY OF STUDY OBSERVATIONS

CODE

- A. D-7,-2,0,1,2,3,4,5,7,8,9,10,14,21,26,28 for each treatment cycle or more frequently as indicated
- B. D-7,D-2,0,2,5,8,14,28 or more frequently as clinically indicated.
- C. D-0,14,21,28
- D. D-7,28,84, every 6 months of1 Stage 3 of trial.
- E. Conducted per FDA regulations on vaccine cells and stored patient specimens in Stage 3.
- F. D-7 baseline data, if the urinalysis shows an active sediment or Cr is elevated by 20% over two determinations then renal scan will be repeated to assess GFR change.
- G. D27, D84 or as clinically indicated by tumor response clinically
- H. D3, D7, D59, D63
- I. D4-7 whenever draining LN is at least two fold greater in one dimension measure
- J. D-2, D0, D26, D28, D82, D84
- K. D0, D28, D56, D84 Then every 2-3 months as clinically indicated or more frequently if clinically indicated
- L. First Cycle Vaccination: pre-injection ,6 hours, 24 hours, 48 hours,120 hours.
- M. D-7, then as clinically indicated
- N. Tumor specific CTL attempted to be cloned from draining lymph nodes, first treatment cycle only.
Tumor specific CTL attempted to be cloned from peripheral blood following vaccination
Tumor specific CTL attempted to be cloned from biopsied draining limb lymph node post-vaccination
- O. D-7, D28, D56, D84, every 6 months

8.0 TUMOR RESPONSE CRITERIA

8.1 Complete Response - Complete disappearance of all clinically detectable malignant disease for at least four weeks. Patients with bony metastases should have normalization of all bone x-rays.

8.2 Partial Response - Greater than or equal to 50% decrease in the sum of the metastatic sites for at least 4 weeks without increase in size of any area of known malignant disease or appearance of new areas of malignant disease.

8.2.a. Measurable (Bidimensional):

Greater than or equal to 50% decrease in tumor size (multiplication of longest distance by the perpendicular distance) for at least 4 weeks without increase in size of any area of known malignant disease or appearance of new areas of malignant disease.

8.2.b. Measurable (Unidimensional):

Greater than or equal to 30% decrease in linear tumor measurements for at least four weeks.

8.2.c. Mediastinal and hilar responses:

- A - On-study width
- B - Normal width
- C - Width after treatment

Partial Response if $\frac{(A-B) - (C-B)}{(A-B)} > 0.3$

8.2.d. Palpable Masses:

Palpable masses that can be measured in only one dimension may be evaluated for response by using this formula:

PR if $\frac{A-B}{A} > 0.3$

8.2.e. Partial Response of Malignant Hepatomegaly

(Liver > 5 cm below costal margin)
Partial response has occurred if the liver measurements in the midclavicular lines (or other consistently used points from the tip of the xiphoid decrease by $\geq 30\%$.

8.2.f. A return of mixed blastic/lytic lesions significantly (> 50%) to normal bone architecture agreed by two independent investigators will constitute a PR.

8.3 Stable Disease:

No significant change in measurable or evaluable disease for at least 4 weeks (> 8 weeks for bone metastases).

No increase in size of any known malignant disease.

No appearance of any known malignant disease.

This designation includes decrease in malignant disease of < 50% or increase in malignant disease of < 25%.

8.4 Progression:

Significant increase in size or number of malignant lesions.

Measurable, bidimensional or unidimensional:

- a. 25% or greater increase over original measurements in the sum of the product of the two largest perpendicular diameters of measurable lesions.
- b. 50% or greater increase in size of product of diameters if only one lesion is available for measurement and was > 2 cm at the initiation of therapy.
- c. 30% or greater increase in linear (unidimensional) tumor measurements for > 4 weeks.
- d. Appearance of any new malignant lesions.

9.0 TOXICITY EVALUATION AND DEFINITIONS

9.1 Definitions

The following toxicity definitions and criteria will be used in this phase I study.

9.1.a. Although this therapy is not a conventional cytotoxic drug, the National Cancer Institute's Common Toxicity Criteria used for phase I evaluation of cytotoxic drugs will be used (Appendices 14.E and 14.F). In addition, dermatologic toxicity measurements of injection site (Appendix 14.E) will define special criteria for toxicity grading at the site of injection.

9.1.b. Subtoxic Dose - a dose that causes consistent changes of clinical parameters of toxicity and might herald toxicity at the next highest Dose Level or with repeated administration. This would include a dose at which consistent Grade 1 toxicities are seen.

9.1.c. Minimal Toxic Dose - the smallest dose of tumor cell vaccine at which one or more of the patients shows consistent, readily reversible clinical toxicity. In general, this will be toxicity of Grade 2 severity, though in some cases (renal, cardiac, neurotoxicity), Grade 1 toxicity will apply.

9.1.d. Maximum Tolerated Dose (MTD) - the highest safely tolerated dose of RCC tumor cell vaccine injected on each treatment arm of the study in Stage 2. This will be at a Dose Level where consistent unacceptable toxicities are observed. Toxicities at the MTD should be reversible and should not subject patients to excessive risks or discomfort. Generally, consistent hematologic, or non-hematological toxicity of Grade 3 or 4 severity will define the MTD, but in some instances, Grade 2 toxicity may also be unacceptable (e.g. renal, pulmonary, neurologic, cardiac, or Grade 4 local injection site toxicities). Conversion of patients to antinuclear-antibody positivity, rheumatoid factor positivity, any clinical evidence of autoimmune disease (including induction of glomerulonephritis) are defined as Grade 3 toxicities.

9.1.e. Recommended Phase II Dose Levels -will be one Dose Level below the MTD if one is approached. Escalation beyond the recommended phase II doses would result in unacceptable, but reversible, toxicity. Considerations such as discomfort to the patient and evidence for any cumulative and/or irreversible vaccine effects (late auto-immunity) will obviously enter into a final determination of the optimal dosages for the phase II studies.

9.1.f. Death on Study - if a patient expires while on study, an autopsy authorized by advanced directive of the patient or by family consent will be sought in every case. Autopsy will be provided at no cost to the family to make histopathological correlations with the clinical data.

9.2 Autopsy Studies

Autopsies will be performed on all patients as a final assessment of any local or systemic toxicity. At autopsy, the skin of the injection sites will be visibly examined, and if any gross lesions are detected, these sites will be sampled for histology. The remaining, non-resected kidney will be thoroughly sampled to rule out that autoimmune renal disease is induced by the renal antigens administered in the renal carcinoma vaccine. All other organs in the body will be thoroughly sampled (per Johns Hopkins Pathology Department routine) to both assess any possible systemic toxicity and to establish proximate and contributing causes of death. Autopsies will be performed by the Pathology housestaff per hospital protocol routine under the supervision of a faculty preceptor and the chief study pathologist, Dr. Audrey Lazenby. If the patient dies outside of the Johns Hopkins Hospital, the body will be transported by the decedent's funeral home to Hopkins. Transportation and autopsy will be performed as above at no cost to the patient's family.

9.3 Management of Toxicities

9.3.a. Local Toxicities:

Tissue destructive toxicities will be evaluated by the Johns Hopkins Dermatology and Plastic Surgery consultation services. Skin care will follow standard practice based on diagnostic testing. If clinically appropriate, in the event of purulent discharge from vaccine sites, cutaneous and sterile saline subcutaneous aspirates for gram stain and culture and antibiotic sensitivity will be obtained to evaluate cellulitis versus GM-CSF induced neutrophilia.

9.3.b. Systemic Toxicities

Fever and chills. All patients will be examined and blood cultured, as well as further evaluated as clinically indicated for fever > 101°F. Fevers may be managed with acetaminophen 650mg po q 4-6 hr. As these patients are uninephric, all non-steroidals and aspirin derivatives will be strictly contra-indicated at all times.

Headache. May be managed by acetaminophen/650 mg po q4h

Nausea, vomiting. May be managed with diphenhydramine (25-50 mg IV). Decadron is strictly contraindicated.

10.0 FORMS TO BE KEPT

On study forms and all clinical data forms will be recorded into the trial data base in the Division of Biostatistics of the Johns Hopkins Oncology Center under the direction of its director, Dr. Steven Piantadosi. Forms to be kept include:

10.1 Case Report Forms will be used in this study. They should be completed and updated by the principal investigators. Forms include eligibility data, history and physical, prior therapy data, concomitant medications, vaccine dose administration, extent of disease, notes, course assessment, flow sheets, skin toxicity evaluation forms, pharmacokinetics for GM-CSF. (Appendix 14.D-14.I).

10.2 Course Assessment (Follow Up Form) will be completed at the end of all treatment courses (Appendix 14.I). The physician will use this form to formally grade drug toxicities following each course according to the NCI Common Toxicity Criteria (Appendices 14.E and 14.F).

IN ADDITION TO COURSE ASSESSMENT FORMS, ALL PHYSICIANS WILL BE REQUIRED TO WRITE AN ENTRY IN THE PHYSICIANS NOTES SECTION OF THE CASE REPORT FORM AT THE END OF EACH COURSE. THIS ENTRY SHOULD SPECIFY:

10.2.a. Hospitalization (if any); Length of Hospitalization

10.2.b. The Number of Febrile Days

10.2.c. Local Wound Care Needs (if any)

10.3 Trial Data Submission - Data is collected and transmitted daily by the Oncology Center Biostatistics Data Manager. This data will be put into the computerized trial data base for immediate access and evaluation of outcomes. Information will also be transmitted to Somatix Therapy Corporation through its clinical trial monitor, and the NIH RAC and FDA through protocol reports per regulations set for human gene therapy clinical trials. The results of this study will be presented to national scientific and medical meetings on a timely basis.

11.0. REPORTING OF ADVERSE DATA

Report by phone to the FDA (301-295-8416) within 24 hours; (recorder after working hours).

- a) All life-threatening events (Grade 4 toxicities) must be reported.
- b) All fatal events while on study.

Written reports to follow within 10 working days to:

Dr. Curt Gunter, or new designate,
Center for Biologics Evaluation and Research
Office of Therapeutics, Research and Review
Food & Drug Administration
8800 Rockville Pike
Bethesda, Maryland 20892

Copies of written reports will also be sent to the NIH Office of Recombinant DNA Activities :

ORDA, National Institutes of Health
Building 31, Room 4B11
Bethesda, Maryland 20892

12.0 INFORMED CONSENT

The informed consent form for this trial is attached as Appendix 14.G. Signed informed consent for participation in this clinical trial will be obtained in conformance with all guidelines of the Joint Committee on Clinical Investigation of the Johns Hopkins Medical Institutions. A patient and family information packet edited by current Johns Hopkins Oncology Center patients and family members has been prepared to help interested patients raise questions and better understand the nature of the trial prior to deciding on whether or not to give consent. (Appendix 14.H)

13.0 FEDERAL SAFETY STANDARDS AND BIOSAFETY REVIEW PROCESS

This phase I trial involves human recombinant DNA therapy. Two levels of federal review must be satisfied prior to commencement of the trial at the Johns Hopkins Oncology Center.

13.1. FDA

The Food and Drug Administration will review and must approve experimental use of the Somatix Therapy Corporation retroviral vector containing the human GM-CSF gene (Appendix 14.B). Production, storage, and use of this replication defective retrovirus in the creation of gene transduced lethally-irradiated renal cell tumor vaccine will conform strictly to FDA regulations for gene therapy studies. The principal investigator will be granted an IND Number. Full documentation of FDA approval and guidelines for use at the Johns Hopkins Oncology Center will be filed with the Joint

Committee on Clinical Investigation of the Johns Hopkins Medical Institutions prior to final institutional approval of this study.

13.2. NIH - Recombinant DNA Advisory Committee

In addition to FDA approval, this phase I trial will be reviewed by the NIH Recombinant DNA Advisory Committee (RAC). This is in conformance with all US human trials of gene therapy. The NIH RAC review will evaluate scientific merit and potential biosafety issues involved in the use of recombinant DNA and retroviral vectors in human subjects. Only upon written NIH RAC approval will this trial proceed. Full documentation of NIH RAC proceedings and documented approval of this trial will be filed with the Joint Committee on Clinical Investigation.

CLINICAL INVESTIGATION CONSENT FORM

The Johns Hopkins Medical Institutions
(The Johns Hopkins Hospital
The Francis Scott Key Medical Center, etc.)

PHASE I STUDY OF NON-REPLICATING
TITLE OF Research Project AUTOLOGOUS TUMOR CELL INJECTIONS
USING CELLS PREPARED WITH OR WITHOUT GM-CSF GENE
TRANSDUCTION IN PATIENTS WITH METASTATIC RENAL
CELL CARCINOMA

Patient I.D. Plate

Explanation of Research Project to Subject:

PURPOSE OF THE STUDY: This is a study of side effects of two forms of a kidney cancer drug that has not been tested in humans for safety. In this study the drugs are shots under the skin of kidney cancer cells made from your cancer. The purpose of this study is to see if important side effects occur after injections of a person's own kidney cancer cells prepared by these new methods. You are being asked to take part in this study because you have a cancer in your kidney. Tests you had show spread of the kidney cancer outside the kidney. Standard treatments with surgery, radiation, immunotherapy, or chemotherapy are not likely to cure you. For this reason kidney cancer patients are asked to think about taking part in this research study.

PROCEDURES: In this study there are two types of kidney cancer cell drugs. The side effects from each preparation are not known. This is the first time either type has been tested in humans. One type of cell drug is made when the patients kidney cancer cells are grown in a sterile dish and in time they make some of a factor called GM-CSF, granulocyte macrophage colony stimulating factor. In the other type of drug, kidney cancer cells are grown the same way, but the human GM-CSF gene is also put into the DNA of the kidney cancer cells. A gene is a piece of DNA with the message in it to tell a cell to make something. In this case the message is to make more GM-CSF. The message is carried into the kidney cancer cells in the dish by a engineered mouse virus which is able to deliver genes into human cancer cells in a dish. In both ways of making kidney cancer cell drugs, the cells are treated with nuclear radiation in the laboratory so they cannot divide anymore. Only after radiation of the cells are they then given as shots. In other words, this cancer cell drug should not grow under the skin as little cancers.

Doctors do not know the side effects after shots from one way of making the cell drug or the other. To study these questions, you will receive one of the two forms of prepared kidney cancer. After surgery, you will be given one of the two kidney cancer cell drug types by randomization, a process similar to a flip of the coin. Neither you nor the doctors taking care of you in the clinic will know which of the two types of cell you will get until after the shots are completed or side effects occur preventing further injections. You will receive cells prepared only one way for the entire study. Your medical care will be the same regardless of which cell preparation injections you receive.

If you take part, you will first have surgery to remove the kidney with the cancer in it. This is where the kidney cancer cells that make up the shots comes from. You will be asked to sign a separate hospital consent form for the operation after having a chance to discuss the surgery. In all patients at Johns Hopkins healthy enough to take part in this study, surgery is routinely offered to patients as a treatment option.

THIS CONSENT FORM CONTINUES ON THE REVERSE SIDE

In other words, you could have the surgery without taking part in this study. The benefits of surgery are to prevent future health problems of spread of the kidney cancer that cause pain, bleeding, and blockage of blood flow from the legs.

To receive the surgery for this study, you will be examined and tested for good health except for the cancer. We will test your blood for HIV. If you have the HIV virus, you cannot take part in this study. If you are a woman of childbearing years you will be tested for pregnancy. If not pregnant, you will be prescribed birth control measures for the study. If you are pregnant, you cannot take part as we do not know what side effects the study could have on an unborn child.

If, after surgery, you do not have kidney cancer under the microscope, your doctors will discuss treatments for the disease that is found in your kidney. After surgery, we will collect cancer cells from your kidney and send them sterilely to a special laboratory. One day after surgery, one of the two types of cell preparation for your cells will be assigned by the randomization -- coin flip. The sterile preparation of kidney cancer cells has been successful 8 times in 10. If the cell preparation is unsuccessful in any way, you will be told this right away and offered other treatment options by your doctors.

Three weeks after your kidney is removed, you will be examined by your doctors and receive further blood and urine tests to see if it is safe to start the shots. If there are complications after surgery which make experimental shots unsafe, you can continue your care in the Oncology Center. If it is medically safe to start, you will be given your first injection of cells under your skin between 28 and 42 days after your surgery. Your first injections will be given to you in the hospital so you can be observed for 24 hours after the injection. If you do not have serious side effects, you can get shots two more times. The shots will be 28 days apart as an outpatient in the Oncology Center. These shots will be given to you on your arm or thigh. Early in the study, you will be given four shots, and if you take part later in the study we will try to increase the dose by giving up to sixteen injections. The number of injections will depend on when you enter the study. If there are not enough of your prepared cells for 16 injections, we will give you as many as possible to use your prepared cells. If your cancer shows signs of shrinking after the injections, you can continue injections until the cells are used up, if no side effects make this unsafe. If your disease worsens, you will be told and other treatment options will be discussed.

During this study, you will receive a number of medical tests to see if the cells are causing side effects or are doing anything to your cancer. Blood tests (about 6 tablespoons per week) and urine will be tested weekly. Skin biopsies will be taken during the first and third time you are injected. At most, this requires the use of local skin numbing medicine as an outpatient. It will make a hole in the top layer of your skin about the size of this letter o and be twice as deep as two quarters are thick. These biopsies are a routine test in dermatology and are unlikely to cause you any complication except a bandaid. They generally take about 5 minutes to do. If a gland in your groin swells to a point your doctors think it

could be helpful for research tests on the effects of the injections on your cancer, they will ask your permission to do a lymph node biopsy. You will be asked to read and sign another consent form for this minor surgery. You may refuse and continue on the study.

You will also receive x-rays such as CT scans or MRIs. After finishing the shots, your doctors will ask you to come for routine checkups to care for your medical needs, and to look for late side effects. If healthy, you may take part in other experimental studies if the injections do not slow or stop your disease.

RISKS AND DISCOMFORTS: You may have side effects from the injections. Common side effects could be skin reactions, pain, redness, swelling, bleeding or ulcers at the injection sites. You may also experience fever, increase in your white blood cell count, swelling in your joints, general swelling over your body, fluid in your lungs or around your heart, weakness, diarrhea, bone pain, low red cell counts, kidney damage, forms of arthritis, or changes in your thyroid. You may have side effects that we do not yet know about. Finally, if you receive your cancer cells prepared with the GM-CSF gene transfer, tests before the shots will check that mouse viruses are not contaminating the cells. In theory, you could get a viral infection from contaminated cells. We do not know what the long-term side effects are in humans.

BENEFITS: These kidney cancer cell shots may stop your cancer, or slow it down. It is unlikely that these shots will cure your cancer. Your taking part in this study will help us to see if this approach to kidney cancer treatment is safe, and this knowledge might help cancer patients in the future.

NEW INFORMATION: Any new information to your doctors during the course of this research that might affect your choice to continue as a research subject will be given to you. This includes new information about the study procedures or physical reactions experienced by other patients involved in this study.

COSTS AND PAYMENTS: The cell treatments, and special research tests will be provided at no charge. All other laboratory, physician, or hospital costs not related to the performance of research during this study will be charged to your insurance company or to you. You will have been informed of the possible cost of treatment on this protocol although this is only an estimate. The Johns Hopkins University, The Johns Hopkins Hospital, and the Federal Government do not have any program to provide compensation to you if you experience injury or other bad effects which are not the fault of the investigators.

ALTERNATIVES TO PARTICIPATION: You do not have to take part in this study. You can quit the study at anytime and continue your care at Johns Hopkins. If you do not want to be in this study, you can still receive your medical care at Johns Hopkins. You may have surgery alone. Other treatments available are interleukin-2 if you have not been treated with this already and other experimental drugs. You can also decide not to receive any treatment at all.

If you sign this form, you are willing to join the research project described to you on the other side of this page. Your doctors, or the investigators, did explain the other kinds of treatment that are available to you and to others. You should ask the principal investigator listed below any questions you may have about this research study. You may ask him/her questions in the future if you do not understand something that is being done. The investigators (or doctors) will share with you any new findings that may develop while you are participating in this study.

The records from this research study will be kept confidential and will not be given to anyone who is not helping on this study, unless you agree to have the records given out. If the study uses a new drug or device that is under the jurisdiction of the Food and Drug Administration (FDA), the FDA government officials may look at the relevant part of your medical records as part of their job to review new drug and device studies.

If you want to talk to anyone about this research study because you think you have not been treated fairly, or think you have been hurt by joining the study, or you have any other questions about the study, you should call the principal investigator, Jonathan W. Simons M.D., at 955-0351, or call the Office of the Joint Committee on Clinical Investigation at 955-3008 or call The Francis Scott Key Medical Center Institutional Review Board for Human Research at 550-1853. Either the investigator or the people in the Committee office or IRB office will answer your questions and/or help you to find medical care for an injury you feel you have suffered. The Johns Hopkins University, The Johns Hopkins Hospital, The Francis Scott Key Medical Center, _____ and the Federal Government do not have any program to provide compensation to you if you experience injury or other bad effects which are not the fault of the investigators.

You may withdraw from the research study at any time. Even if you do not want to join the study, or if you withdraw from it, you will still have the same quality of medical care available to you at Johns Hopkins or the Francis Scott Key Medical Center.

If you agree to join this study, please sign your name below.

**NOT VALID WITHOUT THE
COMMITTEE OR IRB STAMP
OF CERTIFICATION**

**VOID ONE YEAR FROM ABOVE DATE
RPN NO. _____**

Subject's signature
(including children, when applicable)

Signature of Parent or Guardian (when applicable)

Witness to Consent Procedures

Signature of Investigator

Date

*Optional unless subject is illiterate, or unable to sign.

NOTE: Signed copies of this consent form must be a) retained on file by the Principal Investigator; b) kept in patient's medical record; and c) given to the patient.

[illegible]

I hereby give my consent and authorize Doctor _____

RIGHT OR LEFT RADICAL NEPHRECTOMY

Identify and explain in non-medical terms, use no abbreviations

I acknowledge that:

1. The nature and purpose of the operation or other procedure and anesthesia, the risks involved, alternatives and the possibility of complications have been explained to me by Doctor MARSHALL and all my questions, if any, have been answered to my satisfaction. I am aware that the practice of medicine and surgery is not an exact science and I acknowledge that no guarantee has been made as to the results that may be obtained.

2. I consent to the performance of the above-named operation or other procedure and if, during the contemplated operation or other procedure, other conditions are discovered which, in the best judgment of the medical staff of The Johns Hopkins Hospital, require an extension of the original contemplated operation or other procedure or a different operation or other procedure, I authorize and request that the said operation or other procedure be performed.

3. I further consent to the administration of such anesthesia as may be considered necessary or advisable in the judgment of the medical staff of The Johns Hopkins Hospital.

4. Exceptions to surgery or anesthesia, if any, are: NONE
(If "none", so state)

5. I consent to the disposal by authorities of The Johns Hopkins Hospital of any tissues or parts which it may be necessary to remove. I authorize The Johns Hopkins Hospital to retain, preserve, and use for scientific or teaching purposes any tissue or specimens taken from my body.

6. I consent to the admittance of observers, in accordance with ordinary practices of The Johns Hopkins Hospital, to the use of closed-circuit television, the taking of photographs (including motion pictures), and the preparation of drawings and similar illustrative graphic material, and also consent to the use of such photographs and other materials for scientific purposes, provided my identity is not revealed by the pictures or the descriptive text accompanying them.

7. The undersigned acknowledges receipt of a copy of the foregoing consent and authorization to an operation or other procedure.

Witness's Signature _____

Patient's Signature _____

Signature of Physician Securing Consent _____

JHH ID NO.

☐ ☐ ☐ ☐ ☐ ☐

IF PATIENT IS UNABLE TO SIGN OR IS A MINOR, COMPLETE THE FOLLOWING:

Patient (is a minor _____ years of age) or is unable to sign because:

Witness's Signature

Cloest Relative or Legal Guardian's Signature

THE JOHNS HOPKINS HOSPITAL
INFORMED CONSENT SUPPLEMENT

02 STAMP PATIENT'S IDENTIFICATION OR PRINT CLEARLY
 Nursing Unit Clinic Date Initiated - -

03 J.H.H. - History Number

04 Patient's Name (Last, First, M.I.)

I. INDICATIONS FOR THE OPERATION OR OTHER PROCEDURE ARE:

KIDNEY TUMOR

II. MAJOR RISKS OF THE OPERATION OR OTHER PROCEDURE AND ANESTHESIA (including such items as failure to obtain the desired result, discomfort, injury, additional therapy and death):

ANESTHESIA, BLEEDING, INFECTION

III. Alternatives to the proposed operation or other procedure.

STANDARD CHEMOTHERAPY AND RADIOTHERAPY RELATIVELY INEFFECTIVE
 IN TREATMENT OF PRIMARY TUMOR

IV. In instances where a discussion of the above is deemed unwise medical practice, there should be documented a statement to this effect below, stating the reason for this decision (this space may also be used for explanatory diagrams)

Witness's Signature

Patient's Signature

Signature of Physician Securing Consent

JHH ID NO.

The undersigned acknowledges receipt of a copy of the foregoing Informed Consent Supplement for an operation or other procedure this

day of 19

Witness's Signature

Patient, Closest Relative or Legal Guardian's Signature

INFORMATION FOR PATIENTS AND FAMILIES CONCERNING THE KIDNEY CANCER PHASE I STUDY AT JOHNS HOPKINS

INTRODUCTION

The following detailed information might be of interest to you in thinking about taking part in this study. This information might help you and your family members raise further questions for the doctors to explain to you prior to deciding to participate in the study. Cancer patients and family members have helped us with wording.

This study is a phase I study. A phase I study is a testing of side effects of a new experimental form of treatment. Because this treatment is experimental, and evaluation of side effects is the prime aim, no promises can be made that taking part in the study will help you in any way. Your participation is voluntary. You can change your mind at any time, and continue your medical care at Johns Hopkins if you wish. You can choose also to take part later, if your health continues to make you eligible and the study is not finished.

1. SURGERY

You would not have been asked to think about taking part in this study at Johns Hopkins unless there was an abnormal growth (a tumor) in your kidney. You may already have had a needle biopsy by the time you came to see the doctors involved in this study indicating the presence of kidney cancer. Some patients, however, considering this study will not have had a needle biopsy recommended depending on their previous evaluation. Needle biopsies from a long needle inserted from the outside of your body into an area of abnormal growth on a X-ray or CT scan can cause uncontrolled bleeding in some cases. Kidney cancers have many small blood vessels which may not stop bleeding very quickly after the biopsy. Often the tissue diagnosis of the type of abnormal growth in the kidney is most safely made during an operation to remove the cancerous kidney where any bleeding after cancer tissue removal can be controlled by the surgeon.

You are eligible to take part in this study because the doctors have already determined with examination and tests that given your otherwise excellent health surgery has low risks compared to the benefit of removing a cancerous kidney. Even if there were no phase I study, surgery in your particular case would be considered. You are welcome to get a second opinion regarding the surgery if you wish and still take part in the study afterward if you chose. In patients with health such as yours, the mortality rate (percent of all patients who die because of the surgery) at

Johns Hopkins is under 1 patient per 100 in the last 5 years with Dr. Marshall as the surgeon. Dr. Marshall is the surgeon-in-chief for all patients taking part in this study. You will be in the hospital for about 1 week after the operation. After 3 to 6 weeks, almost every patient with your level of health is recovered to resume normal activity including employment. Heavy lifting should be avoided for six weeks.

If the tumor in your kidney is not kidney cancer when studied by the pathologist, treatment will be recommended which is indicated for the type of tumor diagnosed. Only cases of kidney cancer can take part in this study as the technology of cell preparation has only been developed to the point of testing for side effects in cases of kidney cancer. Because patients who take part in this study will be re-injected with their own inactivated kidney cancer cells after they are prepared specially for this study, patients who chose not have surgical removal of the cancerous kidney could not have the cells prepared and could not take part in this study.

Surgery is a standard form of treatment both to make the tissue diagnosis of the abnormal growth and to control local spread of kidney cancer. The benefits to patients after surgery are prevention of local problems which very often, but not always, come from disease spread out of the kidney. Problems prevented by surgical removal of the kidney are pain due to invasion of nearby organs, tumor invasion of major blood vessels causing blocked blood return to the heart, tumor invasion of the liver causing liver failure, and bleeding into the urinary tract from the tumor requiring blood transfusions. Patients who elect not to have surgery at this time can potentially have surgery later if these side effects develop. However, often at such a point surgery is more hazardous than it is now for several reasons your doctors can explain to you.

2. TREATMENT FOLLOWING SURGERY

The X-rays and scans you have had make it unlikely that surgery alone could cure you. There is evidence in your case the cancer has spread to organs beyond the kidney and not all of the cancer cells can be removed from your body by surgery. Only patients with clear evidence of cancer spread outside of the kidney are eligible for this study. The spread of the cancer outside of the original organ is called metastasis. Unfortunately, at least one in three kidney cancer patients have metastases when the tumor is first picked up. In 1993 doctors still do not know how kidney cancer is caused, and do not know how to prevent it.

No widely effective treatment exists for metastatic kidney cancer, called renal cell carcinoma, or RCC for short. Currently, at Johns Hopkins RCC patients are offered (interleukin-2) IL-2 therapy or phase I therapy. Phase I therapy can follow IL-2

therapy if the disease does not respond to IL-2 therapy, and the patient is still healthy enough to receive experimental drug treatment. Phase I therapy is experimental therapy where the study of the **side effects** of the treatment of potential new anticancer drugs is the chief goal; whether or not a phase I treatment works at all against human cancers is not known when a phase I study is started. Safety is studied in humans first before larger numbers of patients get treated to see how well the experimental treatment works against different cancers.

Some patients sent to Johns Hopkins may have already been treated with IL-2 treatment and the tumor either did not shrink or continued to grow despite treatment. Some patients will not have received this treatment or have already chosen to try something else first. IL-2 carries with it a chance of complete shrinkage of the tumor or tumor sites seen on scans in 4 to 6 patients of 100 treated. If this response occurs, the tumor may remain invisible (a remission) for over 12 months or longer. Unfortunately, IL-2 treatment given at the full recommended doses can be extremely dangerous even given in an intensive care unit. The rate of death due to IL-2 treatment of 4 to 6 patients in 100. According to the Food and Drug Administration statistics, the chances of the best benefit from IL-2 (a complete remission) are about as likely as death from the treatment not the cancer. At Johns Hopkins, phase I therapy such as this study may come after or before IL-2 therapy depending on patient health at a given time and patient-physician discussion of risks and benefits. In other words, if you take part in this study and the cancer does not improve, you could then be treated with IL-2 therapy.

3. THREE SCIENTIFIC ASPECTS OF THIS TRIAL FOR TESTING SAFETY

Our group has completed many studies in mice using a strategy stimulating the immune system (the disease fighting system in the human body) to recognize RCC cancer cells and then destroy them. Unfortunately, many treatments which work in mice cancers do not work in human cancers. To move from mouse cancer research to human cancer therapy, safety in humans must first be proved in a phase I study. Three aspects of this experimental cancer treatment strategy are being studied from laboratory experiments.

A. Cancer Cell Preparation for Injection

The first aspect under study for safety is the injection of the patient's own tumor cells to present tumor specific antigens. Antigens are markers on the surface of cells that the immune system can recognize as disease and then send in white blood cells to destroy. These white cells act like "PAC-MEN", gobbling up cancer cells. Our group has developed conditions to grow more human kidney cancer cells outside the body than have ever been injected back into patients to try to stimulate the patient immune system with higher doses of antigens. A safe dose when cells are

prepared in this way is not known. Fortunately, the re-injected cancer cells work just as well inactivated by nuclear radiation in a test tube before injection into mice. A patient's RCC cells before final injection will be treated with nuclear radiation in the laboratory so they cannot divide anymore. In other words these cancer cells should not grow under the skin as little cancers. Instead they are likely to be destroyed by white blood cells that come into the area where they are injected.

B. Granulocyte-Macrophage Colony Stimulating Factor

A second aspect of this trial is the testing for safety of GM-CSF (granulocyte-macrophage colony stimulating factor) by the injected RCC cells carrying the antigens on their surfaces. When expanded in numbers in the technique to grow RCC cells for injection, the GM-CSF factor is secreted by the cells. The new technique of increasing the dose of these cells and injecting them under the skin has not been tested before for safety in human beings. This new technique also has not been studied as a cancer treatment. In the past, tumors were immediately prepared and injected. They were not grown to higher doses but were seen to produce some GM-CSF. In mice the GM-CSF factor may aid the immune system in "kick starting" certain white blood cells to move about the blood stream and to "see" the presence of cancer cells in the body with cancer markers--antigens-- and eliminate them as the system eliminates cells infected with viruses like the flu.

C. Gene Transfer of the Granulocyte-Macrophage Colony Stimulating Factor

The third aspect of this trial is a laboratory finding that mouse RCC kidney cancer cells stimulate the immune system if the GM-CSF gene is put into cancer cells that do not make GM-CSF and then GM-CSF is made and the cells are injected under the skin. A gene is a piece of DNA with the instructions in it to tell a cell to make something. In this case the instructions to make lots of GM-CSF are carried into the cancer cell by a engineered mouse virus which is able to deliver genes well into human cancer cells. The inactivation of the cells with nuclear radiation does not seem to decrease the amount of GM-CSF made.

The National Institutes of Health Recombinant DNA Advisory Committee and Food and Drug Administration have approved pilot studies using injections of human tumor cells genetically engineered to secrete other genes such as the recombinant TNF gene product, interleukin-2 gene product, and the interleukin-4 gene product in patients with advanced cancers including kidney cancer.

This phase I study is the first to employ the GM-CSF gene, and first to use the MFG retroviral vector, the engineered mouse virus GM-CSF gene delivery system.

The cells used to produce the viruses carrying the GM-CSF gene in the laboratory already have passed FDA inspection and have been shown not to make any viruses other than those which are designed to insert a gene of interest into the DNA of a cell of interest. Studies are ongoing at the University of Michigan with this gene transfer system. No side effects from the release of unwanted viruses has been observed so far in patients treated there. If these viruses should be changed in some way, they could in theory cause additional cancers in your body.

In the case of this study the injected cells carrying the transferred GM-CSF gene are destroyed under the skin in the normal process inflammation following injection. This builds in an additional safety feature. Nevertheless, the long-term side effects of all aspects of using the GM-CSF gene transfer are not known and it is important for you to be aware of this.

Humans kidney cancer cells appear different than the mouse kidney cancer model studied which led to this trial. Human kidney cancer cells do make some GM-CSF as they are prepared for injection. The GM-CSF gene transfer increases this by 10 to 20 times, but it is not known what the side effects could be in humans from this when the cells are injected. To study the side effects of **each form of cell preparation**, one without GM-CSF gene transfer and one with GM-CSF gene transfer, patients who take part in this studied will be injected with one preparation or the other. You will not be injected with both because this would confuse the study of which side effects come from which preparation.

D. Study Procedures

If you decide to participate, you will read and sign a separate informed consent form for the surgery as all patients do for nephrectomy at Johns Hopkins. If at surgery, the cancer is diagnosed as renal cell cancer, you will be finally eligible for injections of the prepared cells. Once your own tumor cells are cut out, they will be sent (much like a human organ for transplant) to the laboratories of Somatix Therapy Corporation who are working with your doctors at Hopkins. With the doctors at Hopkins, Somatix Therapy Corporation has received permission from the Food and Drug Administration to prepare the cells for experimental therapy.

Under sterile conditions the kidney cancer cells will be grown and irradiated (X-rayed) so they can no longer grow or spread after injection under the skin. The cells are shipped back to Hopkins right before the time of injection in the Oncology Center. This procedure has been successful in 20 of 22 cases of renal cancer at Johns Hopkins in trial runs at this writing. In other words, you have a 8 to 9 chances in 10 of receiving cells once sent. If for any technical reason the process fails any safety standard set, you cannot receive treatment.

Injections will be given in the Oncology Center. You can begin treatment after recovery from surgery-- between 3 to 6 weeks afterwards if there are no complications from the surgery making experimental treatment unsafe. You will be injected with between 4 to 16 shots every 28 days unless there are side effects. You may be injected up to 3 months. You will get shots in your right thigh, the arm you don't write with, and left thigh, in that order. If there is evidence of any benefit and the dose assigned to you appears safe you can continue until cells run out. If your disease progresses during the study, your doctors will discuss with you other treatment options.

You will be asked to see your doctors at Hopkins for routine check-ups regardless of what happens. The follow-up is first to continue your care and second to study the long-term side effects of the injections.

4. FINANCES AND HOUSING FOR OUT OF TOWN PATIENTS

As in the case of most cancer clinical trials at the Johns Hopkins Oncology Center your insurance company if you have one will be billed for medical costs of care related to kidney cancer patients. These costs, including surgery are about \$19,000. The preparation of the cells and all aspects having to do with research tests will not be billed. Individual patients, depending on patient and family financial circumstances, can receive information and confidential advice on special arrangements on these matters from the Study's Social Worker, Mr. James Zabora at 410-955-2305. Housing and transportation from Johns Hopkins Oncology Center Housing to the Cancer Center for our out of town patients and families can be arranged at a minimal cost depending family circumstances. Mr. Zabora can provide you with this information as well. A patient care representative is on call 24 hours a day as will be your study doctors for concerns or questions.

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants listed on page 2. Begin with the Principal Investigator/Program Director. Photocopy this page for each person.

NAME Jonathan W. Simons, M.D.	POSITION TITLE Assistant Professor of Oncology and Urology	BIRTHDATE (Mo., Day, Yr.) 11/18/58	
EDUCATION (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)			
INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
Princeton University, Princeton, NJ	A.B.	1980	Biochemistry
The Johns Hopkins University School of Medicine, Baltimore, MD	M.D.	1985	Medicine

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

- 1985-86 Intern, Internal Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts
- 1986-88 Junior and Senior Resident, Internal Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts
- 1988-90 Clinical Fellow, The Oncology Center, The Johns Hopkins University School of Medicine, Baltimore, Maryland
- 1989-90 Postdoctoral Fellow, The Oncology Center, The Johns Hopkins School of Medicine, Laboratory of Molecular Genetics, Baltimore, Maryland
- 1990-91 Assistant in Oncology, The Johns Hopkins University School of Medicine, Baltimore, Maryland
- 1991- Active, Full-Time Staff in Oncology, The Johns Hopkins Hospital
- 1991- Instructor in Oncology, The Johns Hopkins University School of Medicine, Baltimore, Maryland
- 1992 Assistant Professor in Oncology and Urology, The Johns Hopkins University School of Medicine, Baltimore, Maryland

Honors

- 1980 Magna Cum Laude in Biochemistry, Princeton University
- 1980-81 Rotary International Foundation Postgraduate Fellowship for the Humanities, University of Kent, England
- 1984-85 Medical Student Research Training Award, National Institutes of Health
- 1985 Henry Strong Denison Award for Medical Research
- 1985 Warfield T. Longcope Prize for Excellence in Clinical Medicine
- 1988-89 American Cancer Society Clinical Oncology Fellowship Award
- 1990 Hecht Fund Award for Cancer Research
- 1990-95 Physician Scientist Award, National Institutes of Health, National Cancer Institute, Award Number K11CA01495-02

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9. Simons, J.W., Oliner, J.D., Cho, K., and Vogelstein, B. SAM 1.1 and JOSH 4.4: Two Highly Informative Restriction Fragment Length Polymorphisms within the Human DCC Gene. *Human Mol. Genet.* 1, 352, 1992.
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HUMAN GENE TRANSFER PROTOCOL

9303-041 Wilmott, Robert W. and Whitsett, Jeffrey, Children's Hospital Medical Center, Cincinnati, Ohio, and Trapnell, Bruce; Genetic Therapy, Inc., Gaithersburg, Maryland; *A Phase I Study of Gene Therapy of Cystic Fibrosis Utilizing a Replication Deficient Recombinant Adenovirus Vector to Deliver the Human Cystic Fibrosis Transmembrane Conductance Regulator cDNA to the Airways.*

Date of RAC Approval: 3/2/1993

Date of NIH Approval: 4/16/1993

"A Phase I Study of Gene Therapy of Cystic Fibrosis Utilizing a Replication Deficient Recombinant Adenovirus Vector to Deliver the Cystic Fibrosis Transmembrane Conductance Regulator Gene to the Airways."

The purpose of this study is to evaluate the safety and efficacy of gene therapy of cystic fibrosis with a replication deficient $\Delta E1, \Delta E3$ deleted adenovirus that directs expression of the normal CFTR mRNA and CFTR protein in mammalian cells. The virus, Av1CF2 will be administered to the upper (nasal) and lower (lobar bronchial) respiratory tract of patients with cystic fibrosis. This protocol is designed to demonstrate 1) the expression of normal CFTR mRNA *in vivo*, 2) the synthesis of CFTR protein and 3) the correction of epithelial cell cAMP dependent Cl^- permeability associated with cystic fibrosis. Potential toxic effects of Av1CF2 will be monitored by measuring clinical, radiographic, physiologic and biochemical parameters. The "pharmacokinetics" or longevity of expression and ability to re-infect the respiratory tract with Av1CF2 will be determined. Systemic and local immunologic consequences of Av1CF2 infection, the time of viral survival, and the potential for recombination or complementation of the virus to produce a replicating virus will be monitored in assessing the safety of the Av1CF2 recombinant virus. The safety and potential risks of exposure of personnel and cohorts of the patients after Av1CF2 administration will be assessed.

Consenting male or female patients of age 18 or greater with FVC >40% and documented homozygote $\Delta 508$ genotype will be recruited from the Cystic Fibrosis Center at Children's Hospital Medical Center, Cincinnati. Patients will be admitted to the Research Center, Children's Hospital after undergoing a two week period of intensive pulmonary toilet including antibiotics, postural drainage, aerosols, mucolytics and/or DNase therapy (pending FDA approval of DNase). Pulmonary function tests will be evaluated pre and post "cleanout." Av1CF2 will then be administered to specific regions of the inferior nasal turbinate, each patient group receiving 5×10^7 , 5×10^8 or 5×10^9 PFU to defined areas of the nasal epithelium. CFTR mRNA, CFTR protein and Cl^- permeability will be assessed in nasal epithelial cells. Three days later, the patient will receive the Av1CF2 in the bronchus of the right or left lower lobe on bronchoscopic examination. Patient groups will receive 10^{10} , 10^{11} or 10^{12} PFU per dose in the lung. Clinical, radiographic, physiologic and cellular evidence of efficacy and toxicity will be assessed post administration. Viral clearance and replication will be assessed and the patient discharged when clear of infectious AV₁CF virus. 7.5 weeks after initial treatment, the patient will be readmitted and the presence of CFTR mRNA and lung function re-assessed. The same dose of virus previously administered to a particular patient will then be readministered to evaluate the potential for reinfection and expression of the CFTR mRNA to the lung epithelium.

The virus, Av1CF2, was constructed by Bruce Trapnell at Genetic Therapy, Inc., Gaithersburg, Maryland, from genetic material obtained from the adenovirus serotype 5. In Av1CF2, human CFTR mRNA is driven by the RSV, (Rous sarcoma promoter-enhancer) and terminates with SV40 poly A signals. The virus harbors deletions in the E1 ($\Delta E1$) and E3 regions ($\Delta E3$) deleted to block replication and to provide insert space for the incoming cDNA respectively. Similar adenoviral constructs have been used to transfer cDNA's for a variety of genes to various mammalian cells *in vitro* and to lung epithelial tissues *in vivo* by this laboratory and by others. It is hoped that these studies will demonstrate the biologic expression *in vivo* and safety of the Av1CF2 vector as a phase I study and support the long term goal of CFTR gene transfer that will lead to physiologically, and ultimately clinically, relevant reconstitution of the abnormalities that cause pulmonary disease in cystic fibrosis.

Cystic fibrosis is an autosomal recessive genetic disease caused by defective salt and water transfer across the cells of the lung and other organs. The disease affects the lungs, digestive system, and sweat glands, as well as other exocrine glands, such as the lacrimal glands and salivary glands. The greatest pathology occurs in the lungs where dysfunction of small airways and recurrent infections cause progressive obstructive pulmonary disease, hypoxemia, and eventually carbon dioxide retention, right ventricular failure, and death. The isolation of the gene for cystic fibrosis (the CFTR gene) has led to the possibility of correcting chloride secretion in the lungs of these patients with normalization of airways function. In this protocol, we propose to evaluate the safety and efficacy of gene therapy in cystic fibrosis using a recombinant adenovirus that is made replication deficient by deletion of the E1 region. It will be used to deliver the CFTR cDNA to the nose, trachea, and one lobe of adult cystic fibrosis patients with mild to moderate disease. We propose to study the safety and efficacy of three different doses of this construct in groups of five patients. The patients will be treated with the ad-CFTR construct and followed for evidence of gene expression, evidence of correction of physiological function of cells, and evidence of improvement in salt transport and mucous clearance in the treated regions of the lung. Patients will be studied for adverse reactions to the virus, duration of the expression of the gene, duration of correction of function, and immunological responses to the construct. After eight weeks, they will be treated again to establish whether the construct will work on a repeated basis and whether there is any toxicity associated with repeated administration.

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Submitted to the NIH Recombinant DNA Advisory Committee

January 4, 1993

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SECTION 1.

INTRODUCTION: CLINICAL AND BIOLOGICAL ASPECTS OF CYSTIC FIBROSIS
AND RATIONALE FOR GENE THERAPY FOR THE PULMONARY MANIFESTA-
TIONS OF CYSTIC FIBROSIS

1.1 Clinical Aspects of Cystic Fibrosis: Cystic fibrosis (CF) is the most common lethal autosomal recessive disease that affects the caucasian population (Boat et.al., 1989; Wood et.al., 1976). Cystic fibrosis has an estimated incidence in the white population of between one in 2,000 and one in 2,500. This disease also affects other ethnic groups, such as the North American black population, with an incidence of one in 17,000, and the native American population with an approximate incidence of one in 80,000. The basic defect of cystic fibrosis is an abnormality of cAMP regulated chloride secretion by epithelial cells, affecting exocrine glands and the lungs. The disease is characterized by abnormal secretions in the respiratory, gastrointestinal, and reproductive tracts and in the sweat glands. The failure of cAMP stimulated chloride secretion by epithelial cells leads to dehydration of secretions which are too viscid or too concentrated. Cystic fibrosis is manifested in the lung by obstructive pulmonary disease with chronic infection, in the gastrointestinal tract by exocrine pancreatic insufficiency leading to malabsorption, in the intestines where abnormal secretions result in small bowel obstruction, in the liver where focal biliary cirrhosis may develop, in the vas deferens resulting in obstructive azoospermia, and by the sweat glands, causing increased secretion of NaCl.

Cystic fibrosis is inherited as an autosomal recessive disease, and the gene is located on the long arm of Chromosome 7 at position 7q31 (Rommens et.al., 1989; Riordan et.al., 1989; Kerem et.al., 1989). At the time of writing, more than 200 mutations have been recognized at this locus (Collins FS, personal communication).

For couples affected by cystic fibrosis, there is a one in four risk of have an affected child in each subsequent pregnancy. It is now feasible to perform limited genetic analysis for prenatal diagnosis, heterozygote detection, diagnosis of atypical cases, and for genetic counseling.

The laboratory diagnosis of cystic fibrosis is based on analysis of sweat collected by the quantitative pilocarpine iontophoresis method of Gibson and Cooke (1959). Samples of sweat for analysis should weigh at least 100 mg; a concentration of chloride greater than 60 meq/l is diagnostic for cystic fibrosis. A false negative sweat test can be found in the presence of edema or hypoproteinemia. A false positive result can be obtained with malnutrition, laboratory error, and various metabolic and endocrine conditions.

The presentation of cystic fibrosis is usually with a combination of diarrhea, recurrent respiratory infections, and failure to thrive. There are many less common presentations of which the most important is small intestinal obstruction by thick inspissated meconium. These children usually present in the first day or two of life with abdominal distention, bile stained vomiting, and delayed passage of meconium. Other presentations of cystic fibrosis include rectal prolapse, nasal polyps, prolonged neonatal jaundice, hypochloremic alkalosis with dehydration, hepatomegaly, and a syndrome of anemia, edema, and hypoproteinemia (Boat et.al., 1989; Wood et.al., 1976).

Although there is a generalized disorder of exocrine secretion in cystic fibrosis, the lungs take the brunt of this disease and account for most of the morbidity and mortality (Wood et.al., 1976). The clinical progression of cystic fibrosis is very variable. Some children have mainly pulmonary disease while others have mainly malabsorption. The disease does not "breed true" in families and subsequent children in one family may have different presentations, different manifestations, and different degrees of severity. It has been reported that the most common mutation for cystic fibrosis, the deletion of phenylalanine at position 508 of the CFTR protein, is strongly associated with pancreatic insufficiency (Kerem et.al., 1990). Other mutations appear to be associated with pancreatic sufficiency and with a milder phenotype. In spite of this variation in phenotype, progressive obstructive pulmonary disease eventually develops in all CF patients. The earliest changes are gas trapping, as shown by an increased residual volume and

increased residual volume to total lung capacity ratio. This is due to increased airways resistance, an abnormality that is present early in the disease. Hyperaeration is often manifested on the chest x-rays of newborn infants with cystic fibrosis. Obstruction to airways produces a typical pulmonary function pattern of airflow obstruction with reduced FEV₁, reduced peak flow rate, and reduced forced expiratory flow from 25-75% vital capacity. Later in the disease, the carbon monoxide diffusion capacity decreases and is a reliable indicator of impaired pulmonary function.

Eventually, the lung disease of CF leads to hypoxemia from intrapulmonary shunting. Hypoxemia leads to increased pulmonary vascular resistance, pulmonary hypertension, and cor pulmonale. Ninety-five percent of CF patients will eventually die from cor pulmonale complicating severe pulmonary hypertension secondary to progressive, generalized derangement of the pulmonary architecture (Wood et.al., 1976). The prognosis for life expectancy in cystic fibrosis has been improving, and the latest data from 1991 show a median survival of 29.4 years (Cystic Fibrosis Patient Registry, 1991: Annual Data Report).

1.2 Lung Disease in Cystic Fibrosis: The lung disease of cystic fibrosis starts early in life, and most infants already have symptoms at the time of diagnosis (Allan et.al., 1980). There appears to be a generalized dysfunction of small airways early in life which is probably the result of defective hydration of secretions, possibly made worse by the increased susceptibility to infection. Some infants with cystic fibrosis develop severe obstructive airways disease, requiring mechanical ventilation (Garland et.al., 1989).

In addition to small airways obstruction from abnormal secretions, cystic fibrosis patients also have an increased susceptibility to lower respiratory infection, especially with the gram negative organism Pseudomonas aeruginosa. The combination of abnormal secretions and infection lead to a necrotizing bronchitis and bronchiolitis with destruction of airway walls, bronchiectasis, and eventually respiratory failure. Inflammation is a major component of this airways disease. There is a profound pulmonary neutrophilia and neutrophil products such as elastase, cathepsin G, and eicosanoids contribute to the inflammation (Berger, 1991). Very high concentrations of interleukin-1 and interleukin-8 have been found in alveolar surface liquid and appear to contribute to the recruitment of neutrophils to the lung (Wilmott et.al., 1990; McElvaney et.al., 1992). Initial pulmonary infections in cystic fibrosis are usually with Staphylococcus aureus. Hemophilus influenzae, E. coli, and klebsiella infections also occur. Inevitably, patients become colonized with Pseudomonas aeruginosa, especially the mucoid strain of Pseudomonas aeruginosa. In some studies, such colonization is associated with poorer survival than in patients not colonized (Wilmott et.al., 1985; Pamela B. Davis, Cystic Fibrosis Foundation Consensus Conference: Clinical Outcomes for Evaluation of New Cystic Fibrosis Therapies, 1992).

1.3 Present Medical Treatment of Cystic Fibrosis: The treatment can be broken into two main areas: treatment of the gastrointestinal disease and treatment of the pulmonary disease. Treatment of the gastrointestinal disease is based on supplements of pancreatic enzymes, supplementation with fat-soluble vitamins, and a high-calorie diet. In patients where weight is not optimum, in spite of this nutritional therapy, enteral feedings may be used via gastrostomy, nasogastric tube, or jejunostomy. In general, the nutritional problems are easier to manage than the progressive pulmonary disease. In patients with advanced pulmonary disease, nutritional status often becomes poor due to repeated infections, poor appetite, and cachexia.

Treatment of the pulmonary disease is based on a regular program to clear secretions, antibiotic therapy, bronchodilator therapy, oxygen administration, and (in the late phases) lung transplantation.

1.3.1 Secretion Clearance Therapy: Physical measures to increase clearance of lower airway secretions have been used to decrease the risk of infection in cystic fibrosis. For more than 30 years, a twice-daily program of chest percussion and postural drainage has been routine therapy and it has been associated with an increase in survival rate. More recently, this approach has been augmented with newer maneuvers, such as positive expiratory pressure breathing (PEP mask), autogenic drainage, and physical exercise.

1.3.2 Antibiotic Therapy: Antibiotic therapy is a mainstay of treatment. Young patients suffer mainly from Staphylococcal infection and may be treated with oral antibiotics, such as cephalexin or Co-trimoxazole. Patients with established Pseudomonas aeruginosa infection frequently require hospital admission for intravenous treatment with anti-pseudomonal drugs. This usually takes the form of combination therapy with a beta lactam drug and an aminoglycoside. These hospital admissions may last 10 to 14 days, or longer, and have been shown to be associated with a decrease in sputum bacterial density and improvement in pulmonary function (Regelmann, 1990). Outpatient treatment of chronic Pseudomonas infection has been hampered by the lack of potent oral anti-pseudomonal agents although the quinolones, such as ciprofloxacin and ofloxacin have shown some promise (Rubio, 1990). More recently, home therapy with intravenous antibiotics has emerged as an effective therapy with financial and social benefits.

1.3.3 Respiratory Support Therapy: Eventually, progressive obstructive pulmonary disease and diffuse bronchiectasis result in hypoxemia, especially at night and with exercise. Supplemental oxygen is used in an attempt to reduce pulmonary hypertension and forestall the development of cor pulmonale. Partial pressures of carbon dioxide (CO₂) in arterial blood do not usually increase until late in the disease. Since the prognosis for successful weaning and extubation of CF patients on mechanical ventilators is poor (Davis, 1978), mechanical ventilation is not usually used to prolong life in cystic fibrosis.

1.3.4 Bronchodilator Therapy: Bronchodilator treatment appears to be helpful in up to 30% of patients with cystic fibrosis. However, bronchodilators need to be used judiciously because some patients have a paradoxical decrease in flow rates following therapy (Landau and Phelan, 1973).

1.3.5 Lung Transplantation: For patients with very advanced lung disease and a poor life expectancy, lung transplantation has been used. Initially, this was developed using heart-lung transplantation (Smyth et.al., 1991). More recently, bilateral single lung transplants have been developed (Pasque et.al., 1990). These have prolonged life in a small number of cases, and the two-year survival rate is 50-60%. However, the numbers of organs available for transplantation are few and it is unlikely that most CF patients will benefit from this mode of therapy.

1.4 New Pharmacological Therapies in Cystic Fibrosis: Many new therapies are being developed to ameliorate the progressive pulmonary disease of cystic fibrosis in the hope of improving life expectancy. These therapies include the use of an aerosolized antiproteases to inhibit neutrophil elastase, such as secretory leukocyte protease inhibitor (McElvaney et.al.,

1992) and aerosolized alpha-1-antitrypsin as another approach to inhibit elastase (McElvaney et.al., 1991). In addition to defective chloride secretion, cystic fibrosis patients also have excessive sodium reabsorption by airway cells leading to excessive absorption of water. This has been treated with aerosolized amiloride to block the sodium channel (Knowles et.al., 1990). The effect of aerosolized amiloride seems to be relatively small and, therefore, administration of tricyclic nucleotides to stimulate chloride secretion has been developed as an adjunctive therapy (Boucher, 1992). At present, these approaches are experimental or in phase I trials.

Much of the viscosity of CF sputum is due to the presence of DNA derived from decaying host neutrophils. It was reasoned that a reduction in viscosity by hydrolysis of such DNA might lead to improved clearance of secretions and improved pulmonary function in cystic fibrosis. Human pancreatic DNase (DNase I) was cloned and produced by recombinant techniques. Children's Hospital, Cincinnati has been involved with Phase II and Phase III studies of the efficacy of this agent with regard to pulmonary function, quality of life, and dyspnea in cystic fibrosis (Fuchs et.al., 1992). Early results with DNase therapy appear promising. Other new approaches to cystic fibrosis are under study and these include other protease inhibitors, anti-inflammatory agents such as alternate-day prednisone and daily administration of anti-staphylococcal antibiotics (Keflex) starting at the time of diagnosis. Although these approaches may lead to amelioration of the progressive lung disease of cystic fibrosis, none of them is likely to result in a complete correction of the pathophysiological process that eventually results in respiratory failure and death.

With the cloning and sequencing of the CFTR gene in 1989 came the possibility of correcting the basic defect in airway cells by somatic cell gene therapy. The respiratory epithelium has a large surface area and can be reached via the airway so it is amenable to gene therapy. The intent of this study is to utilize a replication deficient recombinant adenovirus as a viral vector to deliver the normal CFTR cDNA to airway epithelial cells with the goal of expressing this gene and correcting the chloride channel defect. This should lead to normal hydration of respiratory secretions and normalization of airway function. The technique will be evaluated initially in adults with CF who have mild to moderate lung disease (Crystal et.al., RAC proposal, 1992; Wilson et.al., RAC proposal, 1992; Welsh et.al., RAC proposal, 1992; and the current proposal). However, it is our belief that once the safety of this technique is established, the technique has more promise if used early in life, in the pediatric age group, prior to the development of airway fibrosis, micro-abscesses, and bronchiectasis. Thus, we feel that the development of a pediatric center for gene therapy of cystic fibrosis is an important priority.

1.5 Measurements of Efficacy of Therapy for CF: When designing this protocol, we considered the measures of efficacy that can be used in cystic fibrosis. These include pulmonary function testing, imaging of the chest, growth and development, mucociliary clearance, inflammatory markers, potential difference measurement, *in vitro* studies of epithelial cell function, rates of hospitalization, and quality of life assessments. Nasal and tracheal potential difference measurements and laboratory studies of cells from the treated nostril and the treated lower airways will be used to establish correction of the chloride defect at the level of the membrane and individual cells. It is not anticipated that there will be a significant improvement in oxygen saturation or pulmonary function tests in response to treating one lobe and the trachea. However, it is anticipated that there will be improvement in mucociliary clearance from the treated lobe, and this might lead to correction of ventilation/perfusion abnormalities and, possibly, mucous plugging of small airways. Therefore, we propose to use perfusion scans

using a SPECT (single photon emission computed tomography) camera, thin-section CT of the chest, and mucociliary clearance scans as measures of efficacy.

1.5.1 Perfusion Scans: Macro-aggregated albumin deposits in the first set of capillaries that it encounters. Therefore Tc-99m macro-aggregates of albumin, when given by slow intravenous injection, can be used to measure regional perfusion. The SPECT camera has greater resolution than planar images of the lung (Phipps et.al., 1989) and can provide useful measure of lung function in the treated lobe compared to the control lobe.

1.5.2 Thin-Section CT Scanning: Computed tomography can be used to image segmental and sub-segmental bronchi. The pulmonary parenchyma is imaged well, and the technique is sensitive to slight changes in parenchymal density. There have been several reports that have compared chest radiographs with CT scans in cystic fibrosis, and all have found that CT scanning is more sensitive than chest x-ray in mild cases (Hansell and Stricklan, 1989; Santis et.al., 1991). Thin-section CT scanning of the lungs can be used to detect improvement in mucus plugging or the architecture of the treated lobe.

1.5.3 Mucociliary Clearance Scans: Aerosolized Tc-99m-pyrophosphate, after standard planar imaging, provides an accurate measure the deposition of an aerosol and its rate of clearance from the lungs. Although intersubject variability in measured rates of mucociliary clearance are reported to be high, intrasubject variability is very small (Wilkey et.al., 1980). Subjects in the current study will have baseline measurements of mucociliary clearance in the lobe to be treated, as well as the rest of the lung. Following treatment, changes in clearance in the treated lobe will be compared with its baseline and with the control lobe. Attention will be paid to the possible confounding effects of other medications, such as theophylline, cough clearance, and differences in deposition patterns (William D. Bennett, Cystic Fibrosis Foundation Consensus Conference: Clinical Outcomes for Evaluation of New Cystic Fibrosis Therapies, 1992).

Summary of Clinical Aspects of Cystic Fibrosis: Despite continued progress in the diagnosis, treatment and assessment of cystic fibrosis, morbidity and mortality from the pulmonary complications of the disease remain an enormous burden on patients and their families. The present application seeks to utilize somatic cell gene therapy, by transferring the normal CFTR allele to respiratory epithelial cells, in hopes of correcting the primary biochemical and physiological abnormalities in CF.

1.6 Genetic Basis for Cystic Fibrosis: Cystic fibrosis is now known to be causally related to abnormalities of the gene encoding the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) and more than 200 distinct genetic alterations in the CFTR DNA have been identified and associated with a clinical disorder (Kerem et.al., 1989, Riordan et.al., 1989, Lap Chee Tsui, International CF conference, 1992). The most common defect is caused by the deletion of a nucleotide triplet associated with the loss of phenylalanine residue at position 508 (termed the $\Delta 508$ mutation) that accounts for 70% of CF chromosomes and is associated with clinically severe cystic fibrosis. The isolation of the gene and the identification of the primary structure of the CFTR polypeptide has led to intense efforts to reconstitute the defect by genetic or pharmacologic means.

1.6.1 Structure and Function of the CFTR: The human CFTR (hCFTR) is a 1480 amino acid polypeptide ($M_r=168,021$) with distinct structural domains characterized by distinct hydrophobic regions each corresponding to transmembrane segments (Kerem et.al., 1989). A putative regulatory, or R domain, and two nucleotide binding folds in which resides the $\Delta 508$ mutation (fold 1) are characteristic of the CFTR polypeptide in all species studied to date. The CFTR protein is membrane bound, binds to and hydrolyses ATP, and is both a phosphoprotein and a glycoprotein (Kerem et.al., 1989, Riordan et.al., 1989, Cheng et.al., 1991, Gregory et.al., 1990). Transfer of the full-length cDNA encoding CFTR confers cAMP-dependent chloride pump activity that will complement the chloride transport defect characteristic of cells derived from cystic fibrosis patients (Rich et.al., 1990, Drumm et.al., 1990). Evidence to date strongly supports the role of the CFTR as a chloride transporting protein. Site-specific mutations of the CFTR transmembrane region are associated with alterations in the selectivity of halide ions (Anderson et.al., 1990). However, the strong structural relationship of CFTR to multiple drug resistance proteins (MDR) and other members of the ABC co-transport protein family supports its role as an energy-dependent transporter as well. Abnormalities of routing and processing of CFTR mutant protein are important components of the cellular disorder (Cheng SH, et.al., 1990). The hCFTR cDNA has been transferred to a variety of cystic fibrosis and non-cystic fibrosis cell types wherein the CFTR protein was identified immunohistochemically and associated with the generation of cAMP-dependent chloride transport activity. This activity is distinct from calcium-dependent chloride channels which appear to be present in most cells and distinct from the cystic fibrosis defect. These calcium-dependent regulated chloride channels appear to be intact in cells from cystic fibrosis patients. While the precise manner by which abnormalities of the CFTR cause pulmonary disease and infection are not known, the rapid expansion of our understanding of the molecular aspects of CFTR has provided reagents required for transfer of the CFTR gene to cells, animals and humans for somatic cell gene therapy of CF.

Cystic fibrosis is a disease primarily affecting electrolyte transport in secretory epithelia (Davis, P. 1991). Pulmonary complications of cystic fibrosis represent the major life-threatening abnormality in cystic fibrosis. While growth and function of the lungs in cystic fibrosis patients are generally normal at birth, pulmonary complications begin as early as the first year of life and generally are characterized by thick pulmonary secretions, mucous plugging and the inability to clear respiratory pathogens from the lung, particularly bacterial organisms such as Pseudomonas aeruginosa and Staphylococcus aureus. Chronic pulmonary infection, atelectasis and pneumonia lead to bronchiectasis, and fibrosis, ultimately leading to cor pulmonale and respiratory failure. The importance of the lung complications in cystic fibrosis has focused attention to the CFTR defect in the respiratory epithelium, rather than other organs, for therapy of cystic fibrosis.

1.7 Cells of the Respiratory Epithelium: The respiratory tract begins as an outpouching of the foregut endoderm early in the first trimester of human development and on day 9.6 in the mouse embryo. Initially, the respiratory epithelium consists of undifferentiated columnar epithelial cells which form a dichotomously-branching respiratory tract during the glandular and pseudoglandular period of development. After 4-5 months gestation in the human, during the canalicular period, the epithelial elements of the proximal respiratory tract interact more closely with complex mesenchymal components including blood vessels, smooth muscle and endothelial cells, and begin generating a highly differentiated pattern of cells in the tracheal, bronchial and bronchiolar region of the lung. The differentiation of the proximal respiratory epithelium proceeds until a variety of cell types are produced in the respiratory tract, including squamous,

basal, ciliated, serous, goblet, mucous, non-ciliated-bronchiolar epithelial cells (Clara cells), and neuroepithelial bodies (NEBS). In the human lung, the trachea and proximal bronchi contain numerous tracheal-bronchial glands that are not features of the rodent airway. In the alveolar region, only two epithelial cell types are readily apparent, Type II epithelial cells and Type I epithelial cells. Type I cells are terminally differentiated cells derived from Type II cells that comprise approximately 90-95% of the surface area of the alveolar gas exchange. Type II cells produce pulmonary surfactant and are considered the progenitor cells of the most distal respiratory epithelium. The functions of the distinct cells in the respiratory tract are highly specialized, species specific and subject to developmental regulation. Complex changes in the morphological characteristics of these cells are associated with marked changes in gene expression during development and in response to injury of the airway. Because of this heterogeneity of cellular types and functions it is unclear at present in which cells CFTR defect causes lung disease and whether all or some cells must be treated to effect an amelioration of disease.

1.8 Gene Therapy to the Airway Epithelium: In patients with cystic fibrosis, lung development proceeds normally until after birth, when mucous plugging, air trapping and infection begin to cause chronic lung injury that usually accelerates in the second decade of life. Therapy for the pulmonary complications of cystic fibrosis has been focused on the treatment of the secondary effects of the disease, thought related to abnormal function of the CFTR. Primary therapy may now be considered by transferring the wild-type hCFTR cDNA to the epithelial cells of the respiratory tracts of patients with cystic fibrosis. Biochemical evidence to date confirms the clinical observation that cystic fibrosis is a recessive trait and that expression of the mutant CFTR does not confer a dominant abnormality of cellular function in cells transfected with CFTR. Thus, it is likely that restoration of cAMP-dependent chloride transport, or other more subtle abnormalities of cellular function such as abnormal acidification of intracellular organelles (Barasch et.al., 1991), may be accomplished by transfer of the CFTR gene to cells of the lung. Complicating the therapeutic strategy for gene transfer to the epithelium is the imprecision in our present understanding of the identity of epithelial cells critical to the pathogenesis of the cystic fibrosis defect in the lung. The CFTR gene is expressed at very low levels in the respiratory tract in both normals and individuals with cystic fibrosis (Trapnell et.al., 1991a, Tresize et.al., 1991) and pulmonary epithelial cells express human CFTR at levels below that readily detected by *in situ* hybridization or immunocytochemistry. The precise cellular and intracellular sites of normal and mutant CFTR proteins in the lung have not been clarified at present. While alveolar function is, in general, well maintained in patients with cystic fibrosis, the small airways are involved early in the pathogenesis of the disease as evidenced by the hyperaeration on the chest radiograph commonly seen within the first year of life. The clinical findings in cystic fibrosis, therefore, focus attention to the need to complement the CFTR in the epithelial cell lining of the bronchial and bronchiolar regions of the lung at an early age, before mucous plugging and infection cause irreversible lung disease and make access via the respiratory tract impossible. If cell-specific, temporal and spatial regulation of the hCFTR is required for normal lung function, direct transfer of the gene by plasmid, adenovirus, retrovirus or other vectors, may be greatly complicated and strategies for gene therapy to the respiratory epithelium must consider the possibility that unregulated or imprecisely regulated expression of CFTR itself may be detrimental to lung function, growth or development. However, experiments from this laboratory and others, as outlined later in the present RAC application, support the concept that high level or promiscuous expression of CFTR in

pulmonary epithelial cells does not alter lung growth, development or function making feasible the efforts to transfer the CFTR cDNA in a widespread manner.

1.8.1 Pulmonary Targets for Correction of Cystic Fibrosis by Gene Therapy: While cystic fibrosis involves pathology in a variety of organs, most of the morbidity and early death associated with cystic fibrosis relates to abnormalities of the respiratory tract. The CFTR protein is expressed in epithelial cells in a variety of tissues; however, the epithelial cells of the lung provide the major focus for gene therapy to correct the pulmonary abnormalities associated with the disease. The CFTR mRNA is expressed at relatively low levels in most organs and the distribution of the CFTR protein has not been readily discernable in most tissues by standard immunocytochemical techniques. CFTR mRNA has been detected in lung and other organs by a variety of methods including reverse transcription-PCR (appendix 1, Trapnell et.al., 1991a), S₁ protection (see section 3 of this proposal) and by northern blot hybridization analysis (Riordan et.al., 1989, Tresize et.al., 1991, Fiedler et.al., 1991, Whitsett et.al., 1992). We have recently demonstrated that the CFTR mRNA in mouse lung is confined to epithelial cells of the respiratory tract (Whitsett et.al., 1992). The abundance of the CFTR mRNA is regulated in a developmental manner, being highest in fetal lung (Figure 1), decreasing dramatically prior to birth, and approaching adult levels in the first postnatal weeks (Wert et.al., 1992). In the adult murine lung, CFTR mRNA was found to be distributed relatively homogeneously throughout the respiratory epithelium and was not confined to specific cell types (Figure 1). In the whole mount *in situ* hybridization analysis of the fetal mouse (day 13-15), the CFTR mRNA was observed only in epithelial cells and was most abundant in testes, intestine, salivary duct and in the lung. In the human lung, CFTR mRNA has been detected in lung epithelial cells of the mid-trimester human fetus by *in situ* hybridization analysis (McCray 1992). Low abundance of the CFTR mRNA was detected by reverse transcription-pcr of the RNA from human airway cells obtained by brushing the tracheal-bronchial epithelium during bronchoscopy (Trapnell 1991a). However, in all analyses to date, CFTR protein has not been readily detectable by available antibodies in pulmonary tissues. Present data supports the concept that the hCFTR mRNA is present at relatively low levels throughout the respiratory epithelium, including both distal and proximal regions of the lung. The percentage of cells that need to be corrected to ameliorate lung disease is also not known at present. However, recent work by Johnson et.al., (1992) demonstrated that ion conductance across CF epithelial cell monolayers could be corrected by expression of CFTR mRNA in only 6-10% of the cells comprising the monolayer. While it remains unclear whether correction of CF *in vivo* will require precise temporal-spatial regulation of CFTR expression in the lung, the data at present, demonstrate the presence of CFTR mRNA in virtually all epithelial cell types of the respiratory tract, providing a broad target for expression of the transferred CFTR gene. Since adenovirus is efficient and able to infect a wide variety of pulmonary epithelial cells *in vivo* and *in vitro*, this vector appears reasonably suitable for transfer of the CFTR gene to the respiratory tract.

1.8.2 Strategies for Gene Therapy of the Respiratory Epithelium: Genes may be transferred by a variety of methodologies *in vitro*, however, only a few strategies may be applicable for gene transfer to the respiratory epithelium *in vivo*. At present, these include the use of viral vectors including retroviruses, adenoviruses, adeno-associated viruses, plasmids, liposomes or DNA-carrier complexes. Receptor systems endogenous to the respiratory tract or viral proteins which might aid in the delivery and integration of the DNA into the respiratory tract are also being developed. The respiratory epithelium undergoes very slow regeneration (estimated half

life of epithelial cells is 80-100 days) making retroviral strategies unlikely to succeed since replicating cell targets are extremely rare in the adult lung. Although it would be preferable to permanently transfer the CFTR to the lung, present strategies must consider vectors that efficiently transfer genes to the epithelial cells of the lung and do not pose risks of insertional mutagenesis. To date, lipofection and retroviral methods have not been efficient vectors for gene delivery to the lung *in vivo*.

1.8.3 Animals for the Study of Cystic Fibrosis: Unfortunately, there are no animal models with phenotypic and biochemical features identical to those of human cystic fibrosis. Recently, several groups have produced cystic fibrosis mice (CFTR(-)) by homologous recombination (Snouwaert et.al., 1992, Dorin et.al., 1992). These animals have features consistent with a CF phenotype but, at present, homozygous CF(-) mice generally succumb at an early age from to GI dysfunction and do not develop significant lung pathology. There are no other known animal models with the CF defect with or without pulmonary pathology consistent with cystic fibrosis. The respiratory epithelium of rodents contains cell types virtually identical to those in the human respiratory tract. However, the location and relative abundance of these cells is quite distinct in rodents compared to human lung, and the pathology related to CFTR deficiency in the mouse may be quite distinct from that in human cystic fibrosis. Thus at present there are no animal models with the CF defect that can be utilized to demonstrate amelioration of lung disease by gene transfer of the CFTR. Because of the severe morbidity and mortality associated with CF, and the availability of a technology with the potential for therapeutic benefit, we believe it is appropriate that somatic cell gene therapy in human patients should be considered in spite of the lack of appropriate animal models.

1.8.4 Consideration of Single vs. Repetitive Treatment with Av1CF2: The presently approved protocols for use of adenovirus for delivery of hCFTR (Wilson et.al., RAC proposal, 1992, Welsh et.al., RAC proposal, 1992, and Crystal et.al., RAC proposal, 1992) will test the efficacy of gene transfer with single exposure to virus. Published animal studies have not demonstrated the effectiveness or immunological responses to repeated exposure to the adenoviral-CFTR vectors in test animals. Therefore at present it is unclear whether significant systemic and local humoral, as well as cell mediated responses may be evoked by the virus. Such immunological issues will have to be addressed with all gene transfer strategies, whether viral, plasmid or liposomal. In our protocol we will administer the virus a second time at the same dose given initially. The present application seeks to test whether a second treatment with Av1CF2 will be effective in transferring the CFTR and whether it will initiate a significant humoral or clinical response. The detection of inflammatory cells or a significant clinically detectable inflammatory response in the airway would provide information critical to assessing the likelihood of success in the repeated viral exposure that is expected to be required for lung from therapy of cystic fibrosis. Quantitation of hCFTR mRNA after the first infection (prior to reinfection) will provide information critical to assessment of the length of Av1CF2 direction of hCFTR mRNA in our cystic fibrosis patients.

SECTION 2.

CONSTRUCTION, CHARACTERIZATION AND PRODUCTION OF THE RECOMBI- NANT ADENOVIRAL VECTOR, Av1CF2, EXPRESSING THE HUMAN CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR cDNA

Human CFTR-expressing recombinant adenovirus vectors have been constructed from genetic material derived from human adenovirus serotype 5. The recombinant vector is a generation 1 adenoviral (Av1) vector, termed Av1CF2. Av1CF2 contains (from the left end of the genome) the inverted terminal repeat (ITR) followed by the packaging signal (ψ) and E1a enhancer, and an expression cassette consisting of an rous sarcoma virus promoter (RSV) and a spliced form of the Ad5 tripartite leader (TPL), the human CFTR cDNA coding sequence and an SV40 poly A signals. This is followed by the remainder of the Ad5 genome from position 3328 to the right end except for the deletion contained within the E3 region (Δ E3). Av1CF2 is similar to another human CFTR expressing recombinant adenoviral vector (Av1CF1) which has been described in detail in a protocol previously reviewed and approved by the NIH RAC (Crystal et.al., RAC Proposal, 1992). The principle differences from Av1CF2 include the use of a copy of the Ad5 major late promoter (MLP) instead of the RSV promoter and the use of the E1b poly adenylation signal (Av1CF1 does not contain an SV40 poly A signal) in the expression cassette. For simplicity, only the details of the construction of Av1CF2 will be illustrated; Av1CF1 was constructed using an identical approach (Crystal et.al., RAC proposal, 1992; see below for a details of the genomic organization and a comparison of the minor differences among the vectors used in this study). Because of the similarities in structure and function of Av1CF2 and Av1CF1, they have both been used for preclinical studies in this proposal. However, we will use Av1CF2 in the clinical protocol.

2.1. Clinical Aspects and Molecular Biology of Human Adenoviruses: Adenoviruses (reviewed in Ginsburg, 1984) are small (80-90 nm diameter), non-enveloped viral particles with a spiked, icosohedral morphology and a molecular mass of $175 - 185 \times 10^6$ Da. The genome consists of a linear, double stranded DNA of approximately 36 kb in length which codes for at least 30 different mRNA molecules. The virion is composed of approximately 85 % protein, 13 % DNA, 1 % carbohydrate with no lipid component and has a density in isopycnic gradients of 1.33 g/ml. Serologically, adenoviruses have been classified into 45 - 50 distinct types (Hierholzer, 1992). Based on rodent studies, the various adenoviral serotypes can be further grouped into 5 subgroups based on their ability to induce tumors in newborn hamsters. Thus, group A adenoviruses (serotypes 12, 18, 31) are able to induce tumors in newborn hamsters with the highest frequency, group B (serotypes 3, 7, 11, 14, 16, 21) less frequently while those in group C (serotypes 1, 2, 5, 6), D (serotypes 8 -10, 13, 15, 17, 19, 20, 22-30, 32, 33, 36, 37), and group E (serotype 4) do not induce tumors. It is important to recognize despite the existence of a classification based on tumor formation in newborn hamsters, adenoviruses have never been associated with tumors of any type in humans.

Clinically, adenoviral infection results in several types of illnesses, the nature of which depends on the serotype of the adenovirus involved (Straus, 1984). One of the most common illnesses caused by adenoviruses is an upper respiratory infection resulting in acute febrile pharyngitis accounting for 5 - 15 % of occurrences of the "common cold". The spectrum of common adenoviral associated illnesses also includes kerato-conjunctivitis and self-limited gastroenteritis. Although less common, adenoviruses can also cause pneumonia, bronchitis, hepatitis and cystitis. In immunocompromised individuals, adenoviral infection can result in a life-threatening illness. Thus, while adenoviral infections are very common in the general population, in most individuals they cause only a mild illness. This is supported by the fact that by early adulthood, nearly all (>95 %) normal, healthy individuals have serologic evidence of previous adenoviral infection. Further, fully replicative, wild-type adenoviruses have been used in multiple clinical protocols involving several serotypes administered to a number of patients

via the respiratory or oral route with only mild or no symptoms which consisted of mild upper respiratory symptoms. Importantly, oral administration of live adenovirus (serotypes 4 and 7) has been used successfully in large numbers of military recruits (>5 million individuals) as a vaccination against the natural respiratory infection which commonly occurs in this group (Chanock et.al., 1966; Couch et.al., 1963; Top et.al., 1971a; Top et.al., 1971b). No clinical sequela were noted in individuals in these studies.

The molecular biology of adenoviruses (reviewed in Ginsberg, 1984; Horowitz, 1990a) has long been the subject of active study predominantly as a model for eukaryotic gene expression. The viral genome is organized into several early and late regions each of which plays a specific role within the life cycle of the viral infection (Figure 2). There are four early regions E1, E2, E3, and E4, as well as one major late region with five different principal transcriptional units derived from alternative transcription and splicing of the region. In addition, there are several minor intermediate and/or late regions. The E1 region is of special interest for several reasons. First, it is active immediately, as soon as the viral genome enters the cell nucleus and due to utilization of cellular transcriptional control factors to activate this region. Second, the E1 region is the regulator of all of the other early regions and activation of the E1 region is required for their activation. Third, the E1 region, which is composed of the E1a and E1b genes (Figure 3) is also responsible for cellular transformation which can be demonstrated in cultured cells. The E2 region codes for proteins related to replication of the adenoviral genome (including DNA binding proteins and a viral DNA polymerase). The E3 region codes for polypeptides involved in viral mechanisms which interact with the host cell and effect immune recognition of the infected cell. This region can be safely deleted with no apparent effect on the quantity or kinetics of replication of an otherwise "wild type" adenovirus. The E4 region, together with the E2 region, serve to upregulate the major late transcriptional unit which is transcribed from the major late promoter (MLP). Transcripts from the MLP initiate with a tripartite leader (TPL) which increases the relative efficiency of translation of viral mRNA, a function which appears to be positively influenced by an intermediate to late RNA transcript from the VA₁ region. The major late region codes for almost all of the structural polypeptides including hexon, penton base, fiber, core DNA binding proteins and other minor proteins which form the capsid.

Human adenoviruses undergo a replicative infection cycle in those cells which are permissive for viral DNA replication (human respiratory epithelial cells). The respiratory epithelium of the cotton rat (*Sigmodon hispidus*) is also permissive for human adenoviruses and thus, the cotton rat provides a good model of the human lung for evaluating human adenoviruses. In contrast to retrovirus, there is no obligate proviral phase and, in fact, adenoviruses rarely integrate into the host cell genetic material. Briefly, during an infection, the adenovirus first attaches to the cell membrane by specific cell-surface receptors and is quickly internalized via clathrin coated pits into endosomes. Acidification of the endosome leads to a conformational change within the viral capsid polypeptides which leads to escape of the virion through endosomolysis. The virion, now free in the cytoplasm is transported to the nuclear membrane mediated through nuclear targeting signals within the capsid polypeptides. The adenoviral genome is then transported or injected into the cell nucleus where the genetic program unfolds. The E1 region, being constitutively active, activates E1, E2, and E3 which remain active. After about eight hours, DNA replication begins and subsequently, the major late unit is active leading to formation of approximately 10,000 virions per cell within the nucleus of the cell. Cell death occurs approximately 30 - 40 hours after initiation of infection releasing the intracellular virions.

2.2. Molecular Biology of Δ E1 Recombinant Adenovirus Vectors: The two best studied adenoviral genomes are from serotypes 2 and 5 and complete nucleotide sequences have been determined for each. Most recombinant adenoviral vector gene transfer systems are derived from one or parts of both of these viruses. It has been known for some time that removal of the E1 region will render the adenovirus unable to replicate in many cell types. This is true of all cells which do not produce an E1A-like factor. Such an E1A-like factor can act in trans to restore the replication of Δ E1 viruses back to wild type levels. Replication deficient recombinant adenoviral vectors have been studied for several years with regard to developing their potential for use for *in vivo* human gene therapy. In this context, Perricaudet et.al., (1992) have developed a recombinant adenoviral vector system based on Ad5 carrying deletions in the viral early region 1 (Δ E1) and 3 (Δ E3). The E3 deletion creates space for insertion of an exogenous cDNA as does the E1 deletion. However, as noted above, the E1 deletion also largely inactivates the endogenous genetic program of the virus including its capacity to replicate. Several recombinant human adenoviral vectors have been constructed which are deficient in replication due to E1 region deletions and contain an expression cassette containing the normal human CFTR cDNA (Figure 4). These vectors have minor differences with regard to the structure of the expression cassette and are expected to function similarly. Multiple *in vivo* studies have now shown that these first generation adenoviral (Av1) vectors can efficaciously and safely be used in animals even when large amounts of virus are used (Wilson et.al., RAC Proposal, 1992; Crystal et.al., RAC Proposal, 1992). Studies by Levvero et.al., 1991 have been conducted in non-human primates with an Ad5 based Δ E1, Δ E3 vector expressing the hepatitis surface antigen. Rosenfeld et.al., (1991a) has also carried out studies in cotton rats where similar vectors except expressing either human α 1-antitrypsin or CFTR were successfully transferred to the respiratory airway epithelium *in vivo* with no reported toxicity. Finally, up to 3×10^{11} plaque forming units of recombinant Δ E1, Δ E3 adenovirus has been administered into the lung of a non-human primate with no (Wilson et.al., RAC Proposal, 1992). Thus, it appears that Δ E1, Δ E3 recombinant adenoviral vectors may provide a safe mechanism for *in vivo* gene delivery.

2.3. Construction of Av1CF2: Av1CF2 (Figure 5) was constructed in several steps consisting of routine prokaryotic cloning procedures and a homologous recombination procedure in the cell line 293. First, an adenoviral construction shuttle plasmid (pAvS6) was assembled (see below for details). The human CFTR cDNA insert from the plasmid pBQ4.7 was then removed and inserted into pAvS6 to yield pAvS6-CFTR. The sequence of pAvS6-CFTR was confirmed by direct sequence analysis of both strands and was determined to have the sequence expected (see appendix 7). pAvS6 was then linearized and co-transfected into 293 cells and allowed to recombine with a fragment of the Ad5-derived virus Ad-dl327 to yield Av1CF2. Purified DNA from Av1CF2 was evaluated by Southern analysis to confirm structural integrity (not shown).

2.3.1 Construction of The Shuttle Plasmid pAvS6: The adenoviral construction shuttle plasmid pAvS6 was constructed in several steps using standard cloning techniques and polymerase chain reaction based cloning techniques. First, the 2913 bp BglII, HindIII fragment was removed from Ad-dl327 and inserted as a blunt fragment into the XhoI site of pKSII⁻ (Stratagene, La Jolla, CA) (Figure 6). The orientation of this fragment was such that the BglII site was nearest the T7 RNA polymerase site of pKSII⁻ and the HindIII site was nearest the T3 RNA polymerase site of pKSII⁻. This plasmid was designated pHR.

Second, the ITR, packaging signal (ψ), rous sarcoma promoter, the adenoviral tripartite leader (TPL) sequence and linking sequences were assembled as a block using PCR amplification (Figure 7). The ITR and ψ (sequences 1 - 392 of Ad-dl327 [identical to sequences from Ad5, Genbank accession # M73260]) were amplified (amplification 1) together from Ad-dl327 using primers containing NotI or AscI restriction sites. The Rous sarcoma virus LTR promoter was amplified (amplification 2) from the plasmid pRC/RSV (sequences 209 to 605; Invitrogen, San Diego, CA) using primers containing an AscI site and a SfiI site. DNA products from amplifications 1 and 2 were joined using the "overlap" PCR (amplification 3) with only the NotI primer and the SfiI primer. Complementarity between the AscI containing end of each initial DNA amplification product from reactions 1 and 2 allowed joining of these two pieces during amplification. Next the TPL was amplified (amplification 4) (sequences 6049 to 9730 of Ad-dl327 [identical to similar sequences from Ad5, Genbank accession #M73260]) from cDNA made from mRNA isolated from 293 cells infected for 16 hr with Ad-dl327 using primers containing SfiI and XbaI sites respectively. DNA fragments from amplification reactions 3 and 4 were then joined using PCR (amplification 5) with the NotI and XbaI primers thus creating the complete gene block.

Third, the ITR- ψ -TPL fragment was then purified, cleaved with NotI and XbaI and inserted into the NotI, XbaI cleaved pHR plasmid. This plasmid was designated pAvS6A⁻ and the orientation was such that the NotI site of the fragment was next to the T7 RNA polymerase site (Figure 8).

Fourth, the SV40 early poly A signal was removed from SV40 DNA as a HpaI-BamHI fragment, treated with T4 DNA polymerase and inserted into the SalI site of the plasmid pAvS6A⁻ to create pAvS6 (Figure 8).

The complete nucleotide sequence of the viral and linker elements within pAvS6 has been determined (not shown, but identical to corresponding sequences in pAvS6-CFTR [see appendix 7]).

2.3.2 Construction of pAvS6-CFTR: The cDNA encoding the complete polypeptide of the human cystic fibrosis transmembrane conductance regulator gene (Genbank accession # M28668) was removed from the plasmid pBQ4.7 (Figure 9; kindly provided by L-C Tsui, Hospital for Sick Children, Toronto, Canada) as a PstI fragment, treated with T4 DNA polymerase and inserted into the EcoRV site of pAvS6 (Figure 9). The orientation of this fragment was such that the coding sequence of the CFTR cDNA had its 5' end nearest the T7 RNA polymerase promoter site and its 3' end nearest the T3 RNA polymerase site of the pskII⁻ backbone (not shown). The complete nucleotide sequence of the viral elements, linker sequences and the human CFTR cDNA were determined by automated sequencing (see appendix 7).

2.3.3 Construction of Infectious Av1CF2 Virus: Infectious Av1CF2 recombinant adenovirus was assembled by co-transfection of linearized pAvS6-CFTR plasmid DNA and the large (approximately 35 kb) ClaI restriction fragment of Ad-dl327 genomic DNA in 293 cells (a human kidney cell line containing 11% of the left end of Ad5 Graham et.al., 1977) cells by homologous recombination. To accomplish this, first, the plasmid pAvS6 was linearized by cleavage with KpnI. Then viral genomic DNA was isolated from Ad-dl327 virions by extraction of viral DNA (Hirt, 1967), cleaved with ClaI and the large fragment was isolated by gel electrophoresis. Two micrograms of linearized plasmid DNA and adenoviral DNA were then mixed and cotransfected into 293 cells by the calcium phosphate method (Figure 10). After 16 hr, media containing 1% agarose was overlaid and the cells were incubated in a humidified, 5%

CO₂ environment until plaques appeared (approximately 1 to 2 weeks). Plaques were then picked and intracellular virus was released by 3 successive cycles of freezing and thawing. After clarification by centrifugation, the lysate was used to reinfect 293 cells for a second round of plaque purification. Subsequently, amplification to high titer virus was carried out by successively infecting plates of 293 cells, CsCl purification and concentration of virus and reinfection as described below (Production of High Titer Adenovirus).

2.3.4 Construction of Infectious Av1LacZ4 Virus: Av1LacZ4 was constructed in a fashion similar to Av1CF2 and has an identical general genomic organization. First a nuclear targeted lac Z gene was constructed using standard and PCR based cloning methods. The recombinant gene consists of the first 4 codons from the SV40 large T antigen followed by codons 127 to 147 encoding the SV40 large T antigen nuclear targeting signal (Genbank accession # V01380) and then codons 6 - 1021 of the E. coli LacZ gene (bases 1302 to 4358), Genbank accession #JO1636). This gene fragment was then inserted into the EcoRV site of pAvS6 (Figure 11). The orientation of the gene coding strand with respect to the plasmid pAvS6 was similar to that of pAvS6-CFTR. Final assembly of the infectious, Av1LacZ4 recombinant adenovirus was identical to that of Av1CF2 (not shown).

2.3.5 Construction of Infectious Av1Null1 Virus: Av1Null1 has a genomic organization similar to that of Av1CF1, but does not contain an exogenous cDNA in the expression cassette. The details of its construction have been previously described (Crystal et.al., RAC Proposal, 1992).

2.4. Production of the Recombinant Adenovirus Av1CF2: The strategy for production of the recombinant adenoviral vector Av1CF2 (Figure 12) consists of initially generating a Master Cell Bank (MCB) of 293 cells (a human kidney epithelial cell line containing the left 11% of the Ad5 genome). Cells from the MCB were then be used to generate a stock of high titer Av1CF2 viral stock referred to as the Master Viral Bank (MVB). Both the MCB and MVB will be characterized with regard to performance in production of adenovirus vector and absence of pathogenic contaminants and certified for use in production of biologic material for clinical use under FDA approved procedures for Good Laboratory Practice (GLP).

Aliquots of the MVB will be used to infect aliquots of the MCB as described below to generate "Batches" of crude viral lysate (CVL). Batches CVL will be characterized, purified and stored as lots of purified infectious virus at a concentration of approximately 10¹¹ to 10¹² pfu/ml in a sterile vehicle consisting of 10 mM Tris-HCl, pH 7.4, 1 mM MgCl₂, 10% (v/v) glycerol at -70°C.

All procedures involved in the production, characterization, packaging, labeling and storage of Av1CF2 will be performed in compliance with FDA/GLP regulations. Importantly, quality assessment of the products will be performed at every stage of the production process as outlined below.

2.4.1 Production of High Titer Adenovirus: Batches of Av1CF2 virus will be produced by infecting 30 plates of 293 cells (15 cm diameter, approximately 70 - 80 % confluent) from the MCB with Av1CF2 from the MVB at a multiplicity of infection (MOI) of 10 pfu/cell in IMEM, 2% fetal calf serum, 1% glutamine for 90 min with rocking. Medium will then be supplemented to 10% fetal calf serum and cells will be cultured for 36 - 40 hr. Infected cells will be collected by low speed centrifugation and intracellular virus will be released by 3 cycles of freezing and thawing to generate a batch of CVL containing Av1CF2.

Batches of CVL will be evaluated (see 2.4.4.5 Cell Harvest) and combined for purification as "Lots" of Av1CF2 virus. Cell debris will be removed from the CVL by low speed centrifugation (7000 rpm, 5 min, 4°C). Cleared CVL is then layered onto a cesium chloride step gradient (1.25 g/ml and 1.4 g/ml in 10 mM Tris-HCl pH 7.4, 1 mM MgCl₂) and centrifuged (20,000 rpm, 2 hr, 4°C, SW 28 rotor). The viral band is collected away from other cellular components and re-purified by isopycnic centrifugation. To accomplish this, the virus from the first gradient is layered onto cesium chloride (1.33 g/ml in 10 mM Tris-HCl pH 7.4, 1 mM MgCl₂) and centrifuged (20,000 rpm, 18 hr, 4°C). Intact Av1CF2 virions are collected away from empty or partly filled capsids by side puncture and dialyzed to remove the cesium chloride (10 mM Tris-HCl, pH 7.4, 1 mM MgCl₂, 10% (v/v) glycerol; 4 x 1 L, 1 hr each). Virus is then aliquoted, labeled and stored at -70°C.

2.4.2 Production of the 293 Cell Master Cell Bank: 293 cells were obtained at passage 31 from the American Type Culture Collection. Cells were thawed and seeded into T-175 flasks (Falcon) in IMEM supplemented with 10% fetal bovine serum (parvovirus and mycoplasma screened), 1% glutamine and cultured at 37°C in a humidified 5% CO₂ atmosphere. Medium was changed every 3 days and the cells were split using trypsin/versine (parvovirus and mycoplasma screened) immediately prior to confluency. Sufficient cells were available after 3 passages and were frozen using standard techniques.

2.4.3 Production of the Av1CF2 Master Virus Bank: A Master Virus Bank (MVB) of Av1CF2 vector will be prepared as a seed stock for production of Lots Av1CF2 vector (Figure 12). To accomplish this, Av1CF2 DNA will first be "sterilized" by phenol chloroform extraction, and then collected by ethanol precipitation and re-solubilized in sterile solution. The DNA will then be transfected into an aliquot of 293 cells from the 293 cell (MCB). Infectious virus will be isolated and amplified to high titer by repeated infection and purification cycles using 293 cells from the MCB. This will allow generation of a large amount of viral seed stock (the MVB) which is free of adventitious pathogenic agents.

2.4.4 Quality Assessment and Quality Control: The evaluation and quality control measures used in the production of Av1CF2 virus will occur at each stage of the process. Many of the quality assessment procedures will be performed, independently, by Microbiologic Associates, Inc. or Quality Biotech, Inc. under contract and all procedure will be carried out in compliance with FDA/GLP guidelines. First, the MCB will be characterized and evaluated extensively for the presence of adventitious pathogenic agents (see 2.4.4.3 Master Cell Bank). Av1CF2 virus is thrice plaque-purified to ensure a pure clone of Av1CF2 virus completely free of wild type or parental (Ad-dl327) virus. Second, the MVB generated from the MCB and "sterile" (phenol-chloroform extraction and ethanol precipitation) Av1CF2 DNA will then be characterized structurally, functionally, and for transfer and expression of functional CFTR to cells defective in CFTR function and evaluated extensively for the presence of adventitious pathogenic and other agents (see 2.4.4.4 Master Virus Bank). The MVB will be extensively evaluated for the presence of wild type virus or parental (Ad-dl327 adenovirus (see 2.4.4.4 subsection replication competent adenovirus). Third, each batch of Av1CF2 containing CVL (cell harvest) will be evaluated for sterility prior to purification into Lots of Av1CF2 vector. Fourth, product testing on the final fill material (purified Av1CF2 virus) will consist of functional evaluation, and the presence of adventitious pathogenic and other agents as well as for the presence of wild type virus (see 2.4.4.6 Av1CF2 Production Lots for Clinical Use).

Antibiotics will not be used at any stage during the production of the MCB, MVB or of purified recombinant virus for clinical use to ensure the highest quality material free of contaminating pathogenic organisms.

2.4.4.1 Facilities for Virus Production: Production of recombinant Av1CF2 vector for preclinical and clinical use is done under Good Laboratory Practices" (GLP) in a facility dedicated for this purpose. The room is set up for biosafety level 2⁺ containment. Specifically, the facility (Figure 13) has an outer room for changing into protective outerware and a inner room where viral production occurs. The production room contains a HEPA filtering system. Protective clothing consists of a "bunny" suit, gloves, booties, haircap and a mask.

2.4.4.2 Drug Masterfile and Standard Operating Procedures: A drug masterfile is being prepared for Av1CF2 for submission to the Food and Drug Administration (FDA) concurrent with this protocol. Details of the production, quality assessment, packaging, labeling and storage of the recombinant adenovirus AvV1CF2 intended for clinical use are contained in the drug masterfile and will be on file with the FDA.

2.4.4.3 Master Cell Bank: The Master Cell Bank (MCB) will be evaluated as outlined in recommendations from the FDA's "Points To Consider" for use of mammalian cell lines. The MCB is required to pass all tests and will be discarded if any test is failed. Many of the tests will be conducted in the laboratories at Microbiological Associates (M.A.) Inc.

Karyology Assay; M.A. Inc, Protocol No. 1516.018: This test is designed to confirm the species identity of cultured cells by means of isozyme and cytogenetic analysis.

Tumorigenicity Assay; M.A. Inc, Protocol No. 1514.001: This study utilizes athymic nude mice which are inoculated subcutaneously with cells. Animals are observed for clinical signs, palpated bi-weekly, and submitted for histopathology evaluation of multiple tissues. Any animal which shows a progressing nodule is sacrificed and submitted for histopathologic examination.

Soft Agarose; M.A. Inc, Protocol No. P1516.029: This assay is designed to assess the capacity of cells to form colonies in soft agarose.

Sterility Test; M.A. Inc, Protocol No. 1514.510: This test exceeds 21 CFT 610.12 in utilizing three different broth media and one agar medium for detection of both aerobic and anaerobic bacterial as well as fungal contaminants.

Mycoplasma; M.A. Inc, Protocol No. 1514.102003: This protocol reflects the 1987 "Points to Consider" attachment and US FDA expectations as to mycoplasma testing. The study includes direct inoculation of test article on two types of agar and in the semi-solid broth medium. Broth inoculated with test article is subcultured at three intervals, and incubated aerobically and anaerobically. In addition to the agar and broth cultivation, direct inoculation of the test article on Vero indicator cells and staining with Hoechst stain permits microscopic examination for the presence of mycoplasma.

Electron Microscopy; M.A. Inc, Protocol No. P1516.013: This protocol is designed to evaluate cell cultures for the presence of virus by examination of fixed cell pellets using transmission electron microscopy.

In Vitro Virus Assay; M.A. Inc, Protocol No. 1514.003: A broad spectrum of viral contaminants are detected using an *in vitro* assay with sensitive indicator cells. Indicator cells; including the test article cells, MRC-5 cell line, and Vero cells. Up to three additional cell lines may be required to comply with European regulatory guidelines. Test procedures include direct inoculation of test article as well as blind passage of supernatant fluids at 14 days. Indicator

cells are observed for cytopathic effect for the entire 28 day period. Hemagglutination and hemadsorption are performed twice during the assay.

In Vivo Virus Assay; M.A. Inc, Protocol No. 1514.005002: This test includes the inoculation of adult mice, suckling mice, guinea pigs and embryonated hens eggs via yolk sack and allantoic routes of inoculation. All animals are observed for clinical signs of viral infection. Suckling mice are sacrificed on day 14 for the preparation of a tissue homogenate which is inoculated into a new group of suckling mice. Specific Pathogen Free (SPF) eggs are examined for viability and the blind passage of fluids into new eggs is performed.

Replication Competent-Adenovirus Assay; M.A. Inc, Protocol No. 1514.031002: This assay is designed to detect the presence of human adenovirus in the test article. Indicator cell cultures are inoculated and observed periodically for cytopathic effect (CPE), a passage is performed for enhancement.

Adeno-Associated Virus Hybridization; M.A. Inc, Protocol No. 1516.104017: This assay detects adeno-associated virus DNA present in the test article as determined by Southern hybridization using a ^{32}P -labelled DNA probe.

HIV Co-cultivation Assay; M.A. Inc, Protocol No. P1516.015001: This procedure is designed to detect small amounts of retrovirus that may be present in the test article. To amplify any virus present, selected Peripheral Blood Lymphocytes (PBL) cells are infected, mixed with test article, passaged and analyzed for HIV by observing for cytopathic effect (CPE) and production of viral antigens by antigen capture ELISA.

Hepatitis B; M.A. Inc, Protocol No. P1516.040: This procedure is designed to detect small amounts of Hepatitis B surface antigen that may be present in the test article which would be detected by a third generation ELISA assay.

Cytomegalovirus; M.A. Inc, Protocol No. P1514.030: The test and control articles are directly inoculated onto cell cultures (indicator cells) and examined for 42 days for the appearance of cytopathic effect. Additionally, cells are fixed and examined using immunofluorescent techniques.

Bovine Virus Assay; M.A. Inc, Protocol No. 1514.032001: This study is designed to detect bovine viruses such as Bovine Viral Diarrhea Virus (BVD), Infectious Bovine Rhinotracheitis Virus (IBR), Parainfluenza 3 (PI 3), Bovine Adenovirus (BA), or Parvovirus (BP) using sensitive indicator cells and immunofluorescence and cytopathic effect (CPE) as endpoints.

Porcine Parvovirus; M.A. Inc, Protocol No. P1514.033004: This assay is designed to detect Porcine Parvovirus (PPV) in cell cultures. Test and control articles are analyzed by inoculation of indicator cell cultures and examined for at least 14 days for the presence of CPE. Cultures are also tested for the presence of specific viral antigens using fluorescent antibody techniques.

Parvovirus B-19 Hybridization; M.A. Inc, Protocol No 1516.104017: This protocol is designed to detect parvovirus B-19 DNA present in the test article as determined by Southern hybridization using a ^{32}P -labelled DNA probe.

Epstein Bar Virus (EBV) Probe Assay; M.A. Inc, Protocol No. P1516.104: The purpose of this study is to detect EBV DNA that may be present in the test article as determined by Southern hybridization using a labeled DNA probe. DNA is extracted from the test article cell pellet and blotted onto membranes. Blotted membranes are hybridized to a labeled EBV probe and detected by autoradiography. DNA from Namalwa cells are included as a positive control.

2.4.4.4 Master Virus Bank: The MVB will be evaluated using the procedures outlined below. If any test is not satisfactorily passed, the MVB will be discarded. In addition, the MVB will be extensively evaluated for the presence of wild type or replicative adenovirus in addition to other infectious agents.

Sterility Test; M.A. Inc, Protocol No. 1514.510: See above (2.4.4.3) for description.

Mycoplasma; M.A. Inc, Protocol No. 1514.102003: See above (2.4.4.3) for description.

In Vitro Virus Assay; M.A. Inc, Protocol No. 1514.003: See above (2.4.4.3) for description.

Recombinant Adenoviral Vector-Derived Expression of Functional Human CFTR: The MVB will be evaluated for the presence and function of AvV1CF2 virus by several assays. The total amount of $\Delta E1$ virus will be quantified by the plaque assay (see below) on 293 cells. The efficiency of gene transfer and expression of human CFTR and correction of the epithelial cell Cl^- secretory defect of cystic fibrosis will be evaluated in CFPAC-1 cells by the chloride efflux assay as previously described (Trapnell et. al., 1991b; appendix 1; see below and Figure 21), and by measurement of forskolin-induced potential difference measurements (see below and Figure 22).

Measurement of Forskolin-Induced CFTR-Specific Changes in Electrical Potential: CFPAC-1 cells are cultured on millipore filter supports until confluent in DMEM, 10% fetal bovine serum (FBS) at 37°C in a humidified, 5% atmosphere CO_2 . When the electrical resistance across the membrane is high demonstrating confluence, the cells are infected with Av1CF2 or an irrelevant control adenovirus at several MOIs. After 48 hr, the filter is placed into a Ussing chamber and forskolin - stimulated electrical potential changes are evaluated (see below and Figure 22).

Measurement of $^{36}Cl^-$ Efflux: The chloride efflux assay used has been previously described (Trapnell et.al., 1991b). Briefly, CFPAC-1 cells are seeded on 35 mm plates and cultured in DMEM, 10% fetal bovine serum (FBS) at 37°C in a humidified, 5% atmosphere CO_2 . The cells are 95% confluent within 24 hr and are infected with Av1CF2 or an irrelevant control adenovirus at several MOIs. After 48 hr, the cells are washed twice with bicarbonate-free lactated Ringer's solution, and loaded with $^{36}Cl^-$ at 3 $\mu Ci/ml$ for 2 hr. After extensive washing (x6), cells are evaluated for secretion of $^{36}Cl^-$ into the media by sequentially removing and replacing the Ringer's solution. The aliquots of removed Ringer's lactate solution and finally the cell layer itself are counted by liquid scintillation counting. The data are compiled and represented as the amount (percent) of $^{36}Cl^-$ remaining within cells as a function of time after washing. Forskolin (13 μM) is included in the Ringer's lactate (except the wash) when evaluating cAMP-stimulated $^{36}Cl^-$ efflux (forskolin raises the intracellular cAMP concentration in CFPAC-1 cells).

Replication Competent Adenovirus: The MVB will be evaluated extensively for the presence of contaminating wild type or parental (Ad-d1327) virus using a PCR detection system (Figures 14, 15, and see below section 2.4.4.7).

2.4.4.5 Cell Harvest (Crude Viral Lysate): Aliquots of cells from the Master Cell Bank (MCB) are infected with Av1CF2 virus from the Master Virus Bank (MVB). A resulting "Batch" of Crude Viral Lysate (CVL) will then be evaluated by the assays listed below. Batches of CVL which fail any test will be discarded. Those that pass will be further purified for clinical certification.

Sterility Test; M.A. Inc, Protocol No. 1514.510: See above (2.4.4.3) for description.

Mycoplasma; M.A. Inc, Protocol No. 1514.102003: See above (2.4.4.3) for description.

2.4.4.6 Av1CF2 Production Lots For Clinical Use: After evaluation and certification, Batches of CVL will be combined into Production Lots for final purification of Av1CF2 vector. Each Lot will be evaluated by the assays listed below. Production Lots of Av1CF2 vector "final fill" material will be accepted only if they pass all of the testing procedures listed below.

General Safety; M.A. Inc, Protocol No. P1514.509: This assay is designed to detect extraneous toxic contaminants. Guinea pigs and mice are inoculated with test material and observed for overt signs of ill-health or unusual response.

Sterility Test; M.A. Inc, Protocol No. 1514.510: See above (2.4.4.3) for description.

Limulus Amebocyte Lysate Assay; M.A. Inc, Protocol No. SPGT332070: The purpose of this study is to detect and quantitatively determine the gram negative bacterial endotoxin level of a test article or extract using the Limulus Amebocyte Lysate (LAL) gel-clot method for testing.

Replication Competent Adenovirus: See above (2.4.4.4) for description.

Recombinant Adenoviral Vector-Derived Expression of Functional Human CFTR: See above (2.4.4.4) for description.

2.4.4.7. Evaluation of Av1CF2 For Replication Competent Adenovirus: Purified preparations of Av1CF2 virus will be evaluated for the presence of E1a containing "wild type" virus using quantitative PCR (see appendix 1, Trapnell et.al., 1991a; Rosenfeld et.al., 1992; Crystal et.al., RAC proposal, 1992). In this assay, purified virions are heat denatured and assayed directly by PCR for the presence of E1a sequences to detect wild type or replicative virus. As a control, PCR amplification of the E2b region is performed in parallel. The current level of sensitivity of this detection system is 1 pfu of wild type virus per 10^8 pfu of Av1CF2. However, we are currently investigating ways to increase this sensitivity, but believe that the current levels of sensitivity are appropriate for this clinical study.

Evaluation of the presence of infectious, wild type virus is possible using a plaque assay on indicator cells which do not complement the $\Delta E1$ of Av1 series vectors. However, these assays are difficult to perform, not yet validated for authenticity and are currently of unknown sensitivity and specificity.

SECTION 3.

PRECLINICAL EVALUATION OF Δ v1CF2 SUPPORTING ITS USE IN THE TREATMENT OF THE RESPIRATORY MANIFESTATIONS OF CYSTIC FIBROSIS

3.1 In Vitro Evaluation of Av1CF2-Derived Expression of hCFTR mRNA: Human kidney epithelial cells 293 cells (Graham 1977) were infected Av1CF2; an appropriate 5.2 kb mRNA was detected by northern blot analysis (Figure 16). Likewise, murine respiratory epithelial cells, immortalized by our laboratory from SV40 transformed lung cells, were infected with Av1CF2 at an MOI of approximately 50 pfu/cell. After 48 hours, the RNA was isolated from control and infected cells and subjected to S_1 protection assay designed to detect the endogenous murine and transferred human CFTR mRNA's (Figure 17). The appropriate protected fragments were identified by autoradiography demonstrating the synthesis of the human CFTR mRNA in these cells at levels greatly exceeding that of the endogenous murine CFTR mRNA.

3.2 In Vitro Expression of the CFTR protein by Av1CF2: HeLa cells, lacking readily detectable CFTR protein by immuno-precipitation assays, were infected with Av1CF2. ^{35}S -Methionine labeled cellular proteins were immuno-precipitated with rabbit anti-hCFTR peptide antibody resulting in the identification of a 155 kilodalton protein (Figure 18) migrating as a band consistent with the size of the hCFTR polypeptide as previously demonstrated by Chang et.al., (1990). Immunocytochemistry was utilized to detect hCFTR with monoclonal CFTR antibody (Genzyme, Inc.) after Av1CF2 infection of 293 cells, demonstrating immunostaining of the majority of cells (Figure 19).

3.3 In Vitro Evaluation of Av1CF2 Vector Expression of Functional cAMP Regulated Cl^- Conductance: Biological function of the Av1CF2 directed CFTR protein has been demonstrated by three distinct assays using CFPAC-1 cells. CFPAC-1 cells are pancreatic adenocarcinoma cells that were derived from a patient who is homozygote for $\Delta 508$ mutation. CFPAC-1 cells fail to respond to cAMP. We have utilized 1) epithelial conductance, 2) SPQ fluorescence and 3) Cl^{36} efflux studies, to demonstrate *in vitro* that the CFTR polypeptide directed by Av1CF2 functions in appropriate manner to alter ion transport.

Transepithelial Cl^- Conductance: CFPAC cells were grown to confluence on support matrix infected with Av1CF2, and placed in a Ussing chamber (Figure 20). The conductance was determined over time in the presence and absence of forskolin, ATP, carbachol or ionomycin. Control CFPAC-1 cells failed to respond to forskolin but had detectable responses to carbachol, ATP and ionomycin. In contrast, a marked Cl^- conductance was observed in cells after infection with Av1CF2 virus at 10 MOI.

Cl^{36} Efflux: Studies were performed on CFPAC-1 cells before and after infection with Av1CF2 virus at 200 MOI. Forskolin (13 μM) had no effect on Cl^- efflux in control, Av1LacZ4 cells, but stimulated Cl^- efflux in the Av1CF2 infected cells (Figure 21).

SPQ Assay: SPQ is a Cl^- sensitive fluorescent dye that once loaded into cells, provides an indirect measurement of the intracellular Cl^- . CFPAC-1 cells were infected with Av1CF2 virus and SPQ fluorescence determined over time in the cell suspensions. Control CFPAC-1 cells were not responsive to forskolin, however, after infection with Av1CF2 (10 MOI), a brisk decrease in intracellular Cl^- was observed, consistent with the efflux mediated by the transferred hCFTR (Figure 22).

3.4 In Vitro Transfer of hCFTR mRNA to Airway Epithelium of Cotton Rats: Cotton rats were treated with 3×10^8 pfu of Av1CF2 intranasally in 100 μl sterile media. After 72 hours, animals were sacrificed, the lungs sectioned and *in situ* hybridization performed to identify and compare hCFTR mRNA with the endogenous cotton rat CFTR mRNA (Figure 23). hCFTR mRNA was readily detected in tracheal, bronchial and occasional alveolar cells using ^{35}S -

riboprobes under conditions specific for detection hCFTR mRNA. Similar studies, (not shown) demonstrated β -galactosidase mRNA directed by Av1LacZ4 (6×10^8 pfu) and X-gal staining demonstrating synthesis of bacterial β -galactosidase by the infected cells of the cotton rat. In these animals, dosed intranasally by aspiration, viral infection was not homogenous, being confined to specific lung segments, other segments being entirely free of hCFTR mRNA. Immunohistochemical staining of these sections is in progress at the time of this submission.

Summary of Functional Data From Av1CF2 Adenovirus: Av1CF2 virus directed the synthesis of the human CFTR mRNA producing the hCFTR polypeptide that conferred cAMP dependent changes in conductance, intracellular Cl^- and Cl^- efflux in response to forskolin. These characteristics are consistent with the appropriate function of the transferred hCFTR polypeptide to the CFPAC-1 cells by the Av1CF2 vector.

SECTION 4.

EVALUATION OF THE SAFETY OF THE A_v1CF2 VECTOR MEDIATED GENE
THERAPY FOR CYSTIC FIBROSIS

Gene therapy as a treatment modality for the respiratory manifestations of CF raises several questions with regard to the overall safety of the procedures involved in the clinical protocol. These concerns can be broadly grouped into issues related to 1) routine clinical procedures usually performed in individuals with cystic fibrosis such as phlebotomy, radiologic studies, bronchoscopy and bronchoalveolar lavage; 2) the tissue location and level of expression of the normal CFTR protein; 3) the *in vivo* use of a replication deficient recombinant adenoviral vector to transduce respiratory epithelial cells such as issues of replication of the vector, induction of immune response either from the material administered or from the expression of endogenous viral genes; safety issues for the patient related to alterations in respiratory epithelial cell function other than those related to aberrant levels of CFTR mRNA must also be considered and finally 4) safety issues for the health care workers and patient contacts. Questions related to safety issues in each of these categories will be considered separately below.

4.1 Routine Clinical Procedures Usually Carried Out in CF Patients: Use of standard clinical procedures such as phlebotomy and radiological studies including chest roentgenography, CT scanning, bronchoscopy and bronchoalveolar lavage are commonly carried as part of the standard care of individuals with cystic fibrosis. The safety of each of these procedures is addressed in section 5 of this protocol and in the informed consent and will be discussed with the patient in detail. Standard medical treatment of any complications related to those procedures will be fully available to the subject.

4.2 Safety Considerations Regarding Location and Level of Expression of Normal CFTR Within the Lung: Since the CFTR expression appears to be influenced by both developmental and tissue specific influences, it is possible that unregulated or aberrantly regulated CFTR mRNA could have untoward physiological consequences. The lack of the CF phenotype of heterozygote patients who are carriers for CFTR mutations suggests that potential decrease in copy number of wild type CFTR is not associated with discernible disease and suggesting that modest decreases in CFTR gene expression (if copy number related) is not harmful. At the present time both *in vitro* and *in vivo* studies support the concept that aberrant expression of the CFTR mRNA is not associated with detectable toxicity, see below.

CFTR mRNA has been both permanently and transiently transferred into a variety of normal and CF cells including CFPAC-1 human tracheal grafts (Engelhardt et.al., 1992) CFT-1 cells (Johnson et.al., 1992) and other cells (Tabcharani et.al., 1991; Drumm et.al., 1990) without signs of toxicity. Likewise, adenoviral mediated transfer of CFTR to the lung was not toxic in the cotton rat (Rosenfeld et.al., 1991, 1992). CFTR has been produced at high levels in the breast tissue of transgenic mice without observed toxicity and with concomitant production of readily detectable hCFTR protein in the milk (DeTullio et.al., 1992). In experiments from this laboratory, we have tested the toxicity of CFTR by directing its expression in the lung and other organs using a variety of promoter-enhancer elements obtained from surfactant protein-C (SP-C), CC₁₀ and β -actin genes (Whitsett et.al., 1992a, b). The hCFTR mRNA were expressed at varying levels in several organs of these transgenic mice (Table I). Most of the transgenic mouse lines have been maintained for one to two years during which time we have not observed any evidence of developmental, morphological or physiological abnormalities in these animals. In the SP-C hCFTR transgenic mice (J4) and others, the mRNA or CFTR protein were readily detected in the bronchiolar and alveolar cells (Figure 24) as well as in the testes of transgenic mice. Growth, development and reproduction has been entirely normal in these hCFTR bearing

Table I

Organ Specific Expression of Human CFTR mRNA in Transgenic Mice:
Detection by *In Situ* Hybridization Analysis of Individual Organs¹

Promoter	Organ Evaluated ²						
	Lung	Testes	Ovary	Brain	Uterus	Spleen	Kidney
SP-C	++	++	-	-	-	-	-
CC ₁₀		+	++	+	+	+	+
β-Actin		++	-	-	-	-	-

¹ Mice carrying transgenes consisting of the human CFTR cDNA driven by various promoters as indicated

² Expression in a particular organ is indicated by a "+" or "++" (more abundant expression). Absence of expression in an organ is indicated by "-".

transgenic animals to date (Figure 25).

In summary, evidence to date derived from transgenic mice *in vivo*, cotton rats infected with adeno-CFTR, human respiratory epithelial cells grown on tracheal grafts transfected mammalian cells *in vitro*, all support the safety of expressing the CFTR mRNA at varying levels in a variety of distinct cell types.

4.3 In Vivo Use of a Replication Deficient, Recombinant Adenoviral Vector to transduce Epithelial Cells of the Human Respiratory Tract: Safety concerns in this area are divided into questions regarding 1) toxicity, 2) the potential for vector-induced immune responses, inflammation and tissue damage related to replication of the vector, and 3) alterations in the cell physiology and functions other than those related to CFTR in transduced respiratory epithelial cells and 4) malignancy.

4.3.1 Host Response Following Respiratory Administration of ΔE1, Recombinant Adenovirus: Will the vector express viral genes? If so, will viral proteins expressed within the epithelium or which are part of the initial administration of vector result in a local humoral or cellular immune response? Will a systemic immune response occur following respiratory administration of Av1CF2? Will the patients become immune to the adenovirus vector? What is the relevance, if any, of an E3 vector deletion to immune response in ΔE1 vectors? These related questions will be addressed below.

It is anticipated that the vector may express some viral genes at a very low level. This is based on results observed *in vitro* in cultured epithelial cell lines infected with similar vectors (Ad-CFTR, Av1CF1 (Crystal et.al., RAC proposal, 1992); Ad2/CFTR-1 (Welsh et.al., RAC proposal, 1992); and Ad.CB-CFTR (Wilson et.al., RAC proposal, 1992). In culture, HeLa cells infected with control adenovirus rapidly produce large amounts of hexon protein, a major capsid protein. However, ΔE1 vector infected cells produced only minimal amounts of this protein. Similar results have been observed in Av1CF2 infected Hela cells (not shown) were levels of

expression are equivalent to those of Av1CF1 infected Hela cells. Importantly, however, it is unclear whether this will occur in humans *in vivo* within the respiratory epithelium because infection of freshly isolated explants of human bronchial epithelial cells failed to demonstrate immuno-precipitable hexon protein (the most abundantly expressed adenoviral protein) (Crystal et.al., RAC proposal, 1992).

In regard to the potential development of toxicity or of a sublethal host immune response, the cotton rat provides a useful model for human respiratory epithelium due to the close similarity of the molecular events (with regards to adenovirus) and the respiratory pathology in lung tissues of the cotton rats and humans after infection by wild-type human adenovirus.

Studies in cotton rats have addressed the question of toxicity following administration of $\Delta E1$, $\Delta E3$ adenovirus to the respiratory epithelium *in vivo*. Dose response studies show that the LD50 for Ad5 (wild-type adenovirus) was in the range of 5×10^9 pfu per animal. In contrast, respiratory administration of Av1CF1 of up to 5×10^{10} pfu after in cotton rats resulted in no detectable toxicity after more than 50 days (Crystal et.al., RAC proposal, 1992). In another animal model, administration of Av1CF1 (2×10^{10} pfu/kg) to a single lung of Rhesus monkeys ($n=4$) resulted in no deaths during the three month study period (Crystal et.al., RAC proposal, 1992). Similarly, administration of 3×10^{11} pfu of Ad.CB-CFTR was well tolerated in monkeys (Wilson et.al., RAC proposal, 1992).

The morphological assessment of the respiratory epithelium following pulmonary administration of Av1CF1 to the cotton rat lung (10^9 - 10^{10} pfu/kg) demonstrated a patchy mononuclear infiltrate in the bronchoalveolar distribution focused in the perivascular space (Crystal et.al., RAC proposal, 1992). We have also observed similar pulmonary mononuclear infiltrates in preliminary experiments in cotton rat exposed intranasally to 6×10^8 pfu of Av1LacZ4 or 2×10^8 pfu of Av1CF2 (approximately 4×10^9 per kg). In the monkey model, administration of one of several $\Delta E1$, $\Delta E3$ vectors (Ad-CMV-lacZ, Ad.CB-CFTR) the single lobe of a Rhesus monkey via bronchoscopy demonstrated no pathology at doses of 3×10^{11} pfu/animal (Wilson et.al., RAC proposal, 1992).

In the context of what may be mild local cellular immune response to respiratory administration of $\Delta E1$ adenoviral vectors, a humoral response also may be elicited. For example administration of Av1CF1 to cotton rats via the respiratory route resulted in significant anti-adenoviral antibody titers as early as one week following exposure (Crystal et.al., RAC proposal, 1992) which persisted for up to four months. Studies are still ongoing to determine whether these antibodies were neutralizing to adenovirus-CFTR. In contrast, administration of Av1CF1 to Rhesus monkeys gave a less consistent humoral response (Crystal et.al., RAC proposal, 1992). In other experiments, administration of an adenovirus vector to rhesus monkeys showed an increase in serum neutralization antibodies to adenovirus after 10 days with a fall after 50 days. The titer increased again after repeated administration of the virus. While systemic humoral immunity is clearly elicited by respiratory administration of $\Delta E1$ recombinant adenovirus, it is unclear as to the presence and extent of local humoral immunity or local cellular immunity. There are no data on whether or not these antibodies may cause any hypersensitivity response. Such issues will need to be further evaluated in this protocol and in future protocols.

In summary, in animal models there is evidence of mild inflammation following administration of the vector to the lungs of cotton rats, but not in rhesus monkeys. Importantly, rhesus monkeys given a single dose of an adenovirus-lacZ construct showed no observable changes in behavior, examination, vital signs, CBCs, serum chemistries, arterial blood gases, chest x-rays, and lung compliance (Crystal et.al., RAC Proposal, 1992).

At this time it is unclear whether individuals with CF will behave more like the cotton rats or more like the non-human primates with regard to response to the vector. Human studies are required to define the magnitude of the pulmonary inflammation after receiving this construct. Our protocol will address this question with measurements of BAL cellular composition, and cytokine concentrations. Clinical studies involving administration of wild-type adenovirus (serotypes Ad1, Ad2, Ad3, Ad4, Ad5, Ad6) in doses of up to 10^6 pfu to the human respiratory tract result in only mild self-limited symptoms (Ginberg et.al., 1955, Roder et.al., 1956, Bell et.al., 1956, Couch et.al., 1966, Smith et.al., 1970). Importantly, wild-type adenovirus would be expected to elicit a more robust pathological response than the $\Delta E1$ vectors considered at present, and in these clinical studies the response was mild.

Thus, although toxicity studies raise the issue that respiratory administration of $\Delta E1$, $\Delta E3$ recombinant adenoviral vectors such as Av1CF2 may result in some inflammation, this response appears to be mild and its significance is unclear. It is expected that such a response will be self-limited and should not result in significant pulmonary toxicity, nevertheless, the present protocol will seek to verify the safety of Av1CF2 in the human lung and test for local and systemic immune response. To this end, we will assess anti-adenovirus antibody production in nasal secretions as well as in serum.

Germane to the potential for an anti-adenovirus vector antibody response, is the possibility, or perhaps likelihood, that Av1CF2 vector treatment may be required on a repeated basis. Experiments in cotton rats have now demonstrated that the respiratory tissues can be infected by an $\Delta E1$, $\Delta E3$ recombinant adenoviral vector even in the presence of very high serum anti-adenovirus antibody titers. Further, due to the presence of significant amounts of elastase within the lungs of CF patients, local degradation of potentially neutralizing antibody may limit the utility of this natural defense against the vector in the lungs of CF patients. Thus, it will be important to evaluate the ability to repeat the transduction of human respiratory tissues by Av1CF2 *in vivo* to assess the potential utility of adenoviral-mediated gene therapy for the pulmonary component of cystic fibrosis. This is one of the aims of this study.

The E3 region of Ad5 encodes proteins involved in the immune response of the infected cell (Ginsberg et.al., 1989; Wold et.al., 1991). Specifically, the 19 kDa E3 glycoprotein is involved in down-regulating MCH class I gene product translocation to the cell surface. This appears to have the effect of blocking the natural host defense to the adenovirus infected cells due to a reduction in self-recognition by host immune effector cells. Thus, $E3^+$ vectors might, at least theoretically, be less easily detected by the host immune system than $\Delta E3$ vectors. The absence of the E3 region has been argued as a safety feature in some CFTR expressing recombinant adenoviral vectors (Ad-CFTR, Crystal et.al., RAC proposal, 1992) as has its presence in others (Ad2/CFTR-1, Welsh et.al., RAC proposal, 1992). However, since $E3^+$ gene expression is a function of E1 gene expression, and the E1 region is deleted in all first generation adenoviral vectors for gene transfer, the significance of the of presence or absence of the E3 region in current vectors is unclear with regard to its effect on immunity or persistence of the vector.

4.3.2 Replication of $\Delta E1$ Recombinant Vectors: $\Delta E1$ recombinant adenovirus vectors are replication deficient and can be complemented by wild-type adenovirus. In the absence of wild-type adenovirus under certain conditions where cellular E1a-like factors can act in *trans* complementation might also occur. We have evaluated the replication potential of several Av1 series recombinant adenoviral vectors and compared them to wild-type adenovirus in a variety of settings. First, the potential for replication of Av1CF1, Av1Null1 and Ad5 were evaluated in a cultured epithelial cell line, (HeLa) (Figure 26). HeLa cells were infected with 60 pfu/cell

and evaluated for infectious virus daily for three days using plaque assays. As a control, Ad5 replication competent virus replicated, generating progressively higher titers over this period. In contrast, there was no increase in titers of either Av1CF1 or Av1Null1. To evaluate the potential for viral replication using another, perhaps more sensitive assay, HeLa cells were infected with either Av1CF1 or Av1CF2 or as a control Ad5 at MOI from 10 to 1000 and evaluated by metabolic labelling of DNA using Hirt extraction, restriction endonuclease cleavage, gel electrophoresis and autoradiography. Wild-type Ad5 clearly replicated and yielded virus-specific labelled DNA bands (Figure 27). In contrast, HeLa cells transduced with either Av1CF1 or Av1CF2 at MOI of 10-30 pfu/cell showed no labelled virus-specific DNA bands, even after long exposure of the autoradiogram. However, at high MOI (100, 500, 1000 pfu/cell) some labelling of viral DNA was observed on long exposure on the autoradiogram. Thus, in HeLa cells, at high MOI there is a potential for production of infectious virus, albeit at very low levels if at all, due to the presence of very low levels of viral DNA replication. Importantly, evaluation of the tissue which is the actual target for CF gene therapy in this and other similar protocols has shown that the Δ E1 adenoviral vector Av1CF1 did not replicate in bronchial epithelial cells even at MOIs of up to 1000 pfu/cell (Figure 28). Similarly, Ad-CFTR did not replicate in freshly isolated bronchial epithelial cells (Crystal et.al., RAC proposal, 1992).

4.3.3 Extent of Infection of Bronchial Tissue: The potential for replication was further evaluated in the human tissue which is the actual target of gene therapy in this protocol. To accomplish this, freshly isolated human bronchial epithelium was obtained at bronchoscopy from a normal individual and infected with MOIs from 10 to 1000 pfu/cell with Av1CF1, or with control, Ad5 virus. As expected, Ad5 clearly replicated at all MOIs, while, as a negative control, no labelled viral DNA bands were seen in uninfected cells, (Figure 28). In contrast to Ad5, no labelling could be seen Av1CF1-infected freshly isolated human bronchial epithelial cells at MOI from 10 to 1000 pfu/cell, even after prolonged exposure of the autoradiogram.

Finally, in a preliminary attempt to address the extent of infection of the respiratory epithelium following *in vivo* administration, we have used an Δ E1 marker virus (Av1LacZ4) at 6×10^8 pfu, which is introduced via nasal administration to cotton rats lung tissue and have evaluated the tissue by X-gal staining, (Figure 29). 72 hours following infection, blue staining of nuclei was observed in patchy distribution scattered segmentally in the lung. The punctate pattern of infection suggests that the vector was not being transmitted from cell to cell nor from bronchus to bronchus, consistent with the lack of *in vivo* viral replication. The lack of *in vivo* replication and cell-to-cell spread is supported by *in vitro* infection of several human cell lines demonstrating a clear dose response relationship between MOI and the percent of cells stained with X-gal (Table II and Figure 30).

Three human pulmonary cell lines H441, A549 and WI-38 cells were infected with Av1LacZ4 for 12 to 72 hours, at a multiplicity of infection (MOI) of 10 or 50 viral particles per cell. At each time point cells were stained for β -galactosidase. Values represent the percentage of cells staining positive. No cytopathic changes were seen over the 72 hour time period.

4.3.4 Evaluation of the potential for Av1CF2-induced cellular toxicity: Infection by wild-type or replication competent adenovirus significantly alters the genetic program of the cells evidenced by changes in cell morphology the so-called cytopathic effect (CPE) related to shut off of host cell protein synthesis and eventually leading to cell death. To evaluate the effect of

Table II

In Vitro Transfer of the *E. coli* Lac Z Gene by Av1LacZ4: Evaluation by X-Gal Staining

Time after infection (hr)	Percent of Cells With Blue Nuclear Staining ¹				
	MOI = 10 pfu/cell		MOI = 50 pfu/cell		
	Cell Line		Cell Line		
	H441	WI-38	A549	H441	WI-38
12	8	2	55	29	35
24	17	12	79	41	46
48	21	22	86	54	72
72	27	35	89	65	83

¹Cells were infected with Av1LacZ4 at the MOIs indicated and cultured for the times indicated and then stained with X-gal. Av1LacZ4 expresses nuclear targeted β -galactosidase thus making scoring of positive cells clear and provides a minimum estimation of the efficiency of gene transfer.

Av1CF2 on cell viability, H441, and A549 cells (both human bronchoalveolar carcinoma cells), and human lung WI-38 cells were infected with Av1LacZ4 with 10 and 50 pfu/cell and evaluated for cell viability and morphology. Attachment and growth was normal during the period of transduction and the extent of transduction was noted to be directly related to MOI. Cells transduced with Av1LacZ4 at both MOIs demonstrated normal morphology, no alterations cellular attachment to the dish and no alterations in viability. As stated earlier, evidence of gene transduction was demonstrated by the intense nuclear X-gal staining (Figure 30).

The potential effect of viral transduction on host cells was further evaluated at the molecular level by testing the potential for Δ E1 adenoviral vectors to alter host protein synthesis. HeLa cells were infected with either Av1CF1 or Av1CF2 MOI 50 pfu/cell. Cells were cultured at 37° and humidified 5% CO₂ atmosphere for 48 hours and labelled with [³⁵S]methionine during the last 24 hours of infection. After the labelling period, total cellular proteins were extracted and evaluated by SDS-PAGE (Figure 31), as described by Rosenfeld et.al., 1992. As expected, wild-type Ad5 adenovirus markedly altered the genetic program of cells as evidenced by a severe reduction in the number and quantity of host cell proteins synthesized. In contrast, Av1CF1-transduced cells showed a pattern of host protein synthesis identical to that of un-transduced cells. Similar Av1CF2 transduced and non-transduced cells also showed an identical pattern of protein synthesis. Thus, at the biochemical level, the Av1CF2 vector appears to have no significant negative effects on the genetic program of the host cell at the level of general protein synthesis.

4.3.5 Potential for Malignancy: Could the vector transform respiratory cells and cause malignancy? The risk of Av1CF2 induced malignancy after *in vivo* administration is expected to be extremely low for several reasons. First, there has never been an association of adenovirus infection with malignancy in humans. Despite the high prevalence of infection of the general population by adenovirus there has been no proven association between adenoviral infection of any serotype and any form of human malignancy (Horwitz, 1990, Straus, 1984). Second, clinical administration of wild type adenovirus to an estimated > 5 million military recruits as part of a vaccination protocol has never resulted in an association with malignancy (Chanock et.al., 1966; Couch et.al., 1963; Top et.al., 1971a, Top et.al., 1971c). Third, serotype 5 adenovirus from which Av1CF2 was derived, one of the more common serotypes, is classified as subgroup C, a class that has a lower transforming potential and is non-tumorigenic in rodents (Ginsburg, 1984). Fourth, the transforming genes (E1) have been removed from the vector thus eliminating its transforming potential. Fifth, although one potential mechanism for the induction of host malignancy could occur on the basis of insertional mutagenesis, adenovirus is known to integrate at very low frequencies (Karlsson et.al., 1985, 1986). Therefore, it seems that the risk of malignancy associated with Av1CF2 is very low.

4.4. Risk of Horizontal Transmission of Av1CF2 Vectors From the Treated Patient to Other Individuals: Will the adenovirus vector become infectious? (See also discussion above 4.3.2 Replication of Δ E1 Recombinant Vectors) Once in the epithelial cells of patients, the Δ E1 recombinant adenoviral vector Av1CF2 should not be transmitted to other individuals unless it replicates. It is known that Δ E1 adenoviral vectors can be complemented with respect to replication by E1 genes *in trans* by wild type adenovirus (Figure 32) or cells that contain and express adenoviral E1a gene products (e.g. 293 cells which are used to produce Δ E1 adenoviruses) and also by E1a-like cellular factors. Thus, at least theoretically, the possibility exists for person-to-person transmission if the patient developed an intercurrent infection with wild-type virus after treatment with Av1CF2. While such complementation might provide the factors for generating infectious virus which could lead to transmission, this could only occur if 1) sufficient viral genomic replication occurs, 2) replicated viral genomes are packaged, 3) intracellular-packaged virions are released, and if the extracellular virus survives the hostile milieu present within the CF respiratory tract such that surviving virus is excreted in quantities sufficient to result in secondary infections. Failure of any of these steps would break down the ability for interpersonal spread of the recombinant vector. Thus, while the potential for interpersonal spread is unknown, it is not likely to be an important practical issue with Δ E1 adenoviral vectors.

To insure that complementation by wild virus does not occur, subjects will be screened for the presence of adenovirus infection on admission for this study. Then they will be isolated during the study to prevent adenovirus infection.

Although Ad5 can complement Av1 series Δ E1 adenoviral vectors when co-infected in the same cell, as previously observed for E1⁺, Δ E3 vectors (Berkner and Sharp et.al., 1983), Δ E1, Δ E3 vectors appear to inhibit replication of co-infecting wild-type adenovirus (Figure 32), albeit by an unknown mechanism. Thus, if an individual treated with Av1CF2 were to become infected with wild-type adenovirus, complementation of the E1 defect of the vector might occur. However, it is also possible that the vector may actually limit the wild-type infection. Therefore, it is possible that continued presence of Av1CF2 Ad5 might actually be beneficial, producing a longer duration of effective treatment. Nevertheless, extensive steps will be taken

to minimize the chance of complementation by wild-type adenovirus as described in the human protocols.

4.5. Risk of Vertical or Germline Transmission: Could the Av1CF2 CFTR cDNA be transferred to the germ line? As the integration frequency of wild type adenovirus is very low (Karlsson et.al., 1985, 1986) and that of Av1CF2 even lower due to the absence of replication and lower cellular genomic copy number, germline transmission is unlikely. Further, since most males with cystic fibrosis are sterile (Boat et.al., 1989), at least for males, this risk is even less. Nonetheless, to minimize the risk of germline transmission, males will be required to use a sheath with a spermicidal jelly while participating in the study protocol. Females with cystic fibrosis have reduced fertility, which is approximately 50% normal. To insure that germ line transmission of the transferred gene does not occur in female subjects, the female cystic fibrosis patients being considered for the protocol will need to have documented sterility by tubal ligation, absence of ovaries, or absence of the uterus, or to be using an effective method of birth control which will be maintained during the study.

SECTION 5.

EXPERIMENTAL DESIGN OF THE HUMAN PROTOCOL

5.1 PURPOSE OF THE STUDY: The purpose of this study is to evaluate the safety and efficacy of gene therapy in cystic fibrosis (CF) utilizing a recombinant adenovirus that is made replication deficient by deletion of the E1 region. Recombinant adenovirus (AV₁CF₂) will be used to deliver the human cystic fibrosis transport regulator (CFTR) cDNA to the upper and lower respiratory tracts of cystic fibrosis patients.

Primary Objectives:

1. To establish that adenoviral vector (Av1CF2)-mediated expression of normal CFTR can be achieved in the nose and lower respiratory tract of cystic fibrosis patients.
2. To establish that expression of the Av1CF2-derived normal CFTR cDNA in the nose and lower respiratory tract of CF patients results in correction of transepithelial potential difference and restores cAMP-dependent chloride conductance.
3. To determine the dose response and longevity of Av1CF2-derived CFTR expression after transfer of the normal human CFTR cDNA to the nasal and lower respiratory tract.
4. To evaluate the safety of recombinant adenovirus (Av1CF2) therapy in patients with cystic fibrosis.
5. To evaluate the antibody response to the recombinant adenovirus (Av1CF2).
6. To determine whether expression of the CFTR gene in the lower respiratory tract of CF patients leads to improvement in mucociliary clearance in the treated lobe.
7. To establish the effectiveness and safety of a single repeated administration of Av1CF2 to the lower respiratory tract in CF patients.

Secondary Objectives:

1. To characterize potential toxicity to the lower respiratory tract, after *in vivo* Av1CF2 infection, by assessing pulmonary function (measured by FEV₁), radiographic abnormalities, and lung functions by perfusion scans and mucociliary clearance scans in patients receiving Av1CF2.
2. To characterize the time course of viral survival and/or replication after Av1CF2 treatment.

5.2 RESEARCH PLAN:

5.2.1 Subjects

1. Three distinct groups of five patients each will be studied according to the clinical protocol, increasing the lung dose from 10¹⁰ PFU to 10¹¹ PFU and 10¹² PFU in a sequential manner, proceeding with each higher dose group after acute toxicity studies verify the safety of the regimen. Clinical toxicity of significance at any dose will obviate proceeding with increasing doses. The nasal dose will be 1/200th of the lung dose based on surface area considerations.
2. Cystic fibrosis patients ≥ 18 years old will be eligible.

3. Subject selection: Cystic fibrosis patients who utilize the cystic fibrosis care center at Children's Hospital Medical Center, meet criteria, and are willing to participate and sign a consent form.

5.2.2 Inclusion Criteria

1. Male or female aged ≥ 18 years.
2. Cystic fibrosis documented by a sweat chloride ≥ 60 meq/L by quantitative pilocarpine iontophoresis, homozygous for $\Delta F508$ marker by genetic testing and one finding consistent with the clinical diagnosis of CF. Genetic analysis documenting the $\Delta F508$ mutation will be required.
3. FVC $\geq 40\%$ of predicted value for sex, age, and height. (Value to be obtained on the day of admission, prior to initiation of study.)
4. Oxygen saturation by pulse oximetry (after 10 minutes of rest) $\geq 90\%$ on room air.
5. Female patients will be required to show documentation of sterility or to be using a form of contraception such as the oral contraceptive pill, the sheath, or the diaphragm. The last two methods will only be accepted if used in conjunction with a spermicidal jelly. Male patients will be required to use a sheath in conjunction with a spermicidal jelly.
6. Female patients will have a negative serum pregnancy test.
7. Seropositivity to adenovirus type 5.
8. Patient legally able to provide informed consent.

5.2.3 Exclusion Criteria

1. Hypercapnia defined as $PCO_2 > 50$ mmHg on admission.
2. Investigational drug use within 30 days prior to enrollment.
3. History of hemoptysis requiring interventional therapy such as transfusion, embolization, or pulmonary resection within 180 days prior to admission.
4. Identified focus of infection outside the respiratory tract.
5. Current pneumothorax requiring chest tube.
6. Presence of wild adenovirus infection (by culture or PCR).
7. Abnormal coagulation studies (PT > 13 secs. on vitamin K 5 mg/day, platelet count $< 200,000$, or PTT > 35 secs.).

8. HIV infected individuals.
9. Chronic active hepatitis B surface antigenemia or unstable hepatitis C.

5.2.4 Methods

Study Design: Patients will be admitted to Children's Hospital Medical Center in a negative pressure isolation room on respiratory isolation procedures. See Table III for a summary of the clinical protocol.

Day Minus 30 to Minus 18: Eligible cystic fibrosis patients will be seen in the clinical research center for pre-study evaluations. Fully informed consent will be obtained. The patients will have a baseline history and physical examination. They will also have baseline studies of EKG, chest x-ray, oxygen saturation in room air, pulmonary function tests, nasal brushings and a nasal biopsy, bronchoscopy for bronchoalveolar lavage, cells by brushing and biopsy, adenovirus culture for wild adenovirus infection, PCR for wild adenovirus infection, adenovirus serology, complete blood count, differential cell count and platelet count, prothrombin time and partial thromboplastin time, serum chemistry, urinalysis, sputum culture, genetic analysis for $\Delta F508$, sweat chloride test, an ELISA test for HIV, and (if appropriate) a pregnancy test.

Presence of active adenoviral infection defined by culture or PCR will exclude patients from further study. The BAL fluid will be used for measurement of cellular composition, neutrophil elastase, and interleukin-8 concentrations. These are measurements of airway inflammation.

2-week Cleanout: From days minus 18 to minus 4, the subjects in the study will receive 2 weeks of intravenous antibiotics with the combination of a β -lactam antibiotic and an aminoglycoside based on the results of prior and current respiratory cultures according to the standard divisional protocol. This will be combined with chest percussion and postural drainage, aerosol therapy, and optimization of nutrition. Optimal methods for "cleanout" include use of DNase (if licensed) will be utilized during this period.

Day -3: History and physical examination will be repeated. Baseline chest x-ray, perfusion scan, CT scan of the chest, mucociliary clearance scan, oxygen saturation, and pulmonary function tests will be obtained. Nasal potential difference measurements will be made (see below), to document chloride permeability defect and feasibility of obtaining measurements in each patient. Measurements will be made at 5 identified sites measured along the inferior turbinates of each nostril from the anterior tip of the turbinate. Pharyngeal swabs and stool will be sent for culture for wild type adenovirus, PCR will be performed for wild type adenovirus, and adenovirus serology will be obtained. Complete blood count and differential, PT, PTT, serum chemistry, and urinalysis will be repeated.

Day -2: History and physical examination will be repeated. Subjects will receive nasal treatment with the AvlCF2 vector. The nose will be anesthetized with 2% topical lidocaine and then examined by endoscopy. To facilitate patient cooperation, subjects may receive mild sedation with intravenous midazolam.

The adenovirus-CFTR construct will be applied to an area of approximately 1 square cm in a defined region of the turbinates in a single nostril. The virus will be suspended in a

Table III

Summary of Evaluations

TEST	Day -30 TO -18 TO -4 Screening "Cleanout" Evalns	Rx																Isolation-----¢	
		Nose								Lobe									
		Trachea								Trachea									
		Trachea								Trachea									
History	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
EKG	X																		
CXR	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Scans *		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Oxygen sat.	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
PFT	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Nasal PD																			
Nasal brush and bx.	X																		
Tracheal PD																			
Bronchoscopy: BAL	X																		
-brushings	X																		
-biopsy	X																		
Adenovirus culture **	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
PCR for wild ad.	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adenovirus serology	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CBC & diff.	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
PT & PTT	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Serum chemistry ***	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Urinalysis	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Sputum culture	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Genetic analysis	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Sweat chloride	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pregnancy test	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
ELISA for HIV	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

* perfusion scan, CT scan and mucociliary clearance

** using 293 cells after administration of vector

*** BUN, creatinine, AST, ALT, LDH, serum electrolytes, protein, albumin, Ca, PO₄, glucose, uric acid, alkaline phosphatase, bilirubin, amylase, and triglycerides.

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volume of 50-100 μ L. After remaining in contact with the nasal epithelium for 15 minutes, the nostril will be gently washed with normal saline, and the material aspirated.

Dosages: Each patient group will receive defined doses of the Av1CF2 construct. Doses administered will range from 5×10^7 PFU to 5×10^8 PFU, and 5×10^9 PFU.

Day -1: History and physical examination will be repeated. Nasal potential difference measurements will be made in the treated and untreated nostrils to assess the physiological correction of transepithelial electrical potential difference measurements. The treated area will be identified by endoscopy and compared to the other side.

Day 0: History and physical examination will be repeated. Subjects will receive intravenous sedation and topical anesthesia of both nares (treated and non-treated) of the nose with 2% lidocaine. Epithelial cells will also be collected from the nose by brushing for cell culture. A sterile, disposable bronchial cytology brush will be used to gently brush the nasal mucosa at the location where the recombinant adenovirus was administered. After 10 seconds of brushing, the brush is removed and the cells shaken into 2 ml of sterile culture medium. The cell suspension is then kept on wet ice and placed in tissue culture using standard techniques. *In situ* hybridization, immunochemistry and chloride intracellular measurements will be used to assess expression of the transfected CFTR cDNA.

Following the collection of cells from the nose, the fiberoptic bronchoscope will be introduced. The larynx will be anesthetized with topical 2% lidocaine, and then the patient will be bronchoscoped. PD will be measured in a defined area of the trachea using measurements from the carina to establish a specific location. BAL, brushing, and multiple biopsies (5) will be performed in the lobe that will be treated. The lobe will be selected based on its radiological appearances. We will try to select a lobe that is representative compared to the overall radiological appearance of the chest. The lobe should be dependent during the bronchoscopy to avoid dissemination of the virus to other lobes. All other things being equal, we will select the right lower lobe. Subjects will receive the Av1CF2 vector (10^{10} , 10^{11} , or 10^{12} PFU) in the bronchus in a total of 20 ml sterile fluid. At the end of the procedure, the trachea will be "painted" with a small amount of the suspension, using small volumes and working from peripheral to proximal location. The equivalent lobe on the other side will be used as a control.

Day 3: History and physical examination will be repeated. Nasal potential difference measurements will be repeated, and then subjects will be bronchoscoped again. PD will be measured in the trachea. BAL will be performed for detection of virus, viral DNA, elastase, cytokines, and cellular composition, the latter to assess inflammation. Brushings of the airways will be obtained using disposable bronchial cytology brushes. Bronchial biopsies will be obtained for histology, *in situ* hybridization of the CFTR mRNA, immunochemical and physiological detection of the wild type CFTR. The treated lobe and the control lobe will be studied. Chest x-ray, perfusion scans, CT scans, mucociliary clearance scans, oxygen saturation, and pulmonary function tests will be repeated to detect any improvement in the function of the treated lobe. CBC and differential, PT, PTT, serum chemistry, and urinalysis will be repeated.

Day 7: History and physical examination will be repeated. Chest x-ray, scans, oxygen saturation, and pulmonary function tests will be repeated. Pharyngeal swabs and stool will be obtained for adenovirus culture using the permissive cell line (293) which supports the growth

of Av1CF2. PCR will also be performed for wild adenovirus, and blood will be obtained for adenovirus serology, CBC and differential, PT, PTT, serum chemistry, and a urinalysis.

Day 14: History and physical examination will be repeated. Oxygen saturation and pulmonary function tests will be repeated. Adenovirus and serum for antibody will be collected. Blood will be taken for CBC and differential, PT, PTT, and serum chemistry. A urinalysis will be performed.

If the recombinant virus is no longer present as free viral particles in secretions, the culture on 293 cells will be negative. If patients have cleared the recombinant virus, they will be discharged at this point. If the virus has not been cleared, the admission will be continued and cultures will be checked every 3 days until the recombinant virus has been eliminated.

The criteria for discharge will be a negative culture for the recombinant adenovirus on 293 cells and a negative PCR for wild adenovirus. The cell line 293 is a human renal cell line that contains 11% of the left end of adenovirus 5, and it complements the replication deficient adenovirus construct, permitting its growth in culture. This system will culture free viral particles present in secretions but not intracellular viral particles (Av1CF2).

Following discharge, patients will be asked to remain at home and to avoid contact with sick individuals and young children. They will also be asked to avoid crowded places such as movie theaters and shopping malls. In this way, we will decrease the risk of a wild adenovirus infection that could, in theory, complement the replication deficient virus and produce an infectious agent.

Day 21: History and physical examination will be repeated. Oxygen saturation and pulmonary function tests will be repeated. Blood will be drawn for adenovirus serology, CBC and differential, PT and PTT, serum chemistry. Urinalysis will be obtained.

Day 28: Patients will be readmitted to the clinical research center for studies. History and physical will be obtained. Chest x-ray will be repeated. Oxygen saturation and pulmonary function tests will be performed. Patients will be sedated and nasal potential difference, nasal brushing and biopsy, tracheal potential difference, bronchoscopy for BAL, brushings and biopsy will be performed as previously. Pharyngeal swabs and stool will be obtained for culture, and PCR will be performed for wild adenovirus infection. Blood will be drawn for adenovirus serology, CBC and differential, PT and PTT, and serum chemistry. Urinalysis and sputum culture will be obtained.

Days 35, 42, 49: Patients will be seen at weekly intervals to check on their progress. History and physical will be obtained. They will have oxygen saturation and pulmonary function tests performed. Adenovirus cultures will be repeated on pharyngeal cultures and stool, PCR for wild adenovirus will be performed. Blood will be obtained for adenovirus serology, CBC and differential, PT and PTT, serum chemistry. Urinalysis will be obtained.

Day 55: Patients will be readmitted into isolation in the clinical research center to prepare for a repeat treatment to the same lobe with the Av1CF2 construct. On admission, they will have a history and physical. Chest x-ray, scans, oxygen saturation, and PFTs will be repeated. Adenovirus culture and PCR will be repeated. Blood will be drawn for adenovirus serology, CBC and differential, PT and PTT, and serum chemistry. Urinalysis will be repeated. Sputum culture will be obtained.

Day 56: Patients will be given intravenous sedation with midazolam and will have repeat measurements of nasal PD, nasal brushings and biopsy, tracheal PD, and bronchoscopy for BAL, brushings, and biopsy. Following the examination, subjects will receive the Av1CF2 vector at the same dose level of their previous dose (10^{10} , 10^{11} , or 10^{12}) in the bronchus (the same site previously treated on day 0) in a total of 20 ml of sterile fluid. At the end of the procedure, the trachea will be "painted" with a small amount of the suspension, using small volumes and working from peripheral to proximal location. The equivalent lobe on the other side will be used as a control.

Day 59: History and physical examination will be repeated. Chest x-ray, lung scans, oxygen saturation and pulmonary function tests will be repeated. Patients will be sedated for measurements of tracheal PD. Bronchoscopy will be performed for BAL, brushings, and biopsy. Adenovirus culture will be performed on BAL and pharyngeal swabs. Blood will be drawn for adenovirus serology, CBC and differential, PT and PTT, and serum chemistry. Urinalysis will be repeated.

Day 63, 70, and 77: Patients will be kept under observation at weekly intervals. They will have weekly history and physical, oxygen saturations, pulmonary function tests, cultures, and blood work. Chest x-rays and lung scans will be repeated according to Table III. If the culture for the recombinant adenovirus using 293 cells is negative on day 70, patients will be discharged following the same protective precautions as were followed after the first administration of the vector.

Day 84: Patients will be readmitted to the clinical research center. They will have a history and physical, chest x-ray, oxygen saturation and pulmonary function tests. Following sedation they will have measurements of nasal potential difference, nasal brushings and biopsy, tracheal potential difference, and bronchoscopy for BAL, brushings and biopsy. BAL, pharyngeal cultures, and stool will be sent for adenovirus culture using the permissive cell line. PCR will be repeated for wild adenovirus infection. Blood will be obtained for adenovirus serology. CBC and differential, PT and PTT, serum chemistry, and urinalysis will be obtained.

Days 91, 98, and 105: Patients will be seen at weekly intervals for history and physical, oxygen saturation and pulmonary function tests, cultures, PCR for wild adenovirus, and blood tests as shown in Table III.

Day 112: This is the final day of the study. Patients will be seen again in the clinical research center for history and physical, chest x-ray, lung scans, oxygen saturation, and pulmonary function tests. Following intravenous sedation they will have measurements of nasal PD, nasal brushing and biopsy, and tracheal PD. Bronchoscopy will then be performed for BAL, brushings, and biopsy. Adenovirus culture will be performed with BAL, pharyngeal swabs, and stool. PCR for wild adenovirus will be performed. Blood will be obtained for adenovirus serology, CBC and differential, PT and PTT, and serum chemistry. Urinalysis will be obtained. Sputum culture will be repeated.

Throughout the experimental protocol we will maintain routine CF care; namely, chest physical therapy 3 times per day, pancreatic enzymes, multivitamins, and routine aerosol therapy.

Method for Measurement of Transepithelial Potential Difference Across the Nasal Epithelium: It has been reported that patients with cystic fibrosis have more negative values of transepithelial electrical potential difference across the nasal and bronchial epithelium (Knowles et.al., 1981). Values are -24.7 ± 0.9 mV in the nose of healthy controls compared to -53.0 ± 1.8 mV (SEM) in older CF patients (greater than 5 years old).

Measurement of potential difference is minimally invasive and safe. The reference bridge is polyethylene PE240 tubing filled with 3mmol KCl in 4% agar connected to a sterile needle placed subcutaneously on the forearm. The reference bridge is connected through a Calomel cell to a volt meter. The exploring bridge is a small polyethylene tube perfused with saline solution at 0.2 ml/minute. This is connected to a 3mm of KCl bridge and then a Calomel cell. The electrical potential difference between the exploring and reference electrode is measured by a low impedance volt meter. The output of the volt meter is connected to a strip chart recorder. The exploring electrode (the fine polyethylene tubing) is used to measure the transepithelial potential difference by applying the tip of the electrode to the mucosa under direct vision. The measurement of potential difference takes 5-10 minutes, and must be performed prior to the application of topical anesthetics. We will also use chloride selective microelectrodes to determine the driving force for chloride.

Chest X-ray: The patients will receive AP and lateral chest x-rays 12 times during the performance of this protocol (Table III).

Method for Perfusion Scans, CT Scans, and Mucociliary Clearance:

1. Aerosol Inhalation for Mucociliary Clearance

Approximately 0.250 mCi of aerosolized Tc-99m-pyrophosphate will be administered by nebulization (inhalation through the oropharynx with use of nose plugs). The amount of activity will be estimated by the gamma camera count rate. Standard dynamic gamma camera images will be obtained for 45 minutes. Quantitative measurements of clearance of radioaerosol will be made for both the right and left lung large airways. Particle size will be approximately 3 microns.

2a. High resolution single photon emission tomography (SPECT) of the lungs.

With patient supine, 2 mCi of Tc-99m-macroaggregated albumin (MAA) will be administered by slow intravenous injection. SPECT data will be acquired for 20 minutes using the Trionix TRIAD high resolution SPECT camera, with reconstruction as transverse, coronal and sagittal orthogonal sections. A single fiducial marker will be used for later section alignment.

2b. Thin section CT (1.5 mm slice thickness, 10 cm slice interval) will be acquired using a GE 9800 CT scanner. Two slices will be acquired at each of 3 levels. A single fiducial marker again will be used.

2c. SPECT sections will be matched. Each will be graded on a 1-4 scale for abnormality (CT qualitatively 1 = normal, 4 = most abnormal). SPECT qualitatively (1 = 75-

100% of maximal perfusion, 4 = 0-25% of maximal perfusion). Ratings for comparable lung regions will be compared.

Pulmonary Function Tests: Oxygen saturations will be measured in room air using a Nellcor pulse oximeter. Spirometry, flow volume loops, and lung volumes by whole body plethysmography will be measured on the Medical Graphics 1070/1085 system. This system is located in the divisional pediatric pulmonary function laboratory (ASB-4). The mouthpiece, tubing, and protective filter will be discarded after use. The protective filter (PALL Protec filter model PF-30S) has a pore size of 0.1μ and protects the spirometer from contamination.

The PFT technicians will wear eye shields, fine particle respirators, gowns, and gloves while performing the test.

Bronchoscopy: Bronchoscopy will be performed using a 4.7 mm Olympus fiberoptic bronchoscope. Patients will receive intravenous sedation with midazolam and Demerol according to standard hospital procedures. The nose will be anesthetized with 2% lidocaine, the larynx and vocal cords will be anesthetized with 2% lidocaine, and the lower airways will be anesthetized with topical 1% lidocaine. This is a routine clinical procedure in the division; 60-70 fiberoptic bronchoscopies are performed by the division each year without significant complications. The safety of this procedure is well established. Airway surface fluid and cells will be obtained by bronchoalveolar lavage. Briefly, the tip of the bronchoscope will be positioned in the same segment of the lobe each time and 20cc of sterile preservative-free saline will be injected and then rapidly aspirated and collected in a suction trap. This will be performed a total of 5 times. The usual volume of fluid recovered is 40-50% of that administered. At the end of the procedure, the other airways will be checked and suctioned free of any retained fluid.

Airway brushings will be obtained using sterile, disposable biopsy brushes and the cells from these brushes will be removed into sterile culture medium by shaking.

Bronchial biopsies will be obtained via the channel of the bronchoscope using fine (2mm) biopsy forceps.

The instruments used for this study will be specially purchased and will not be in general use.

Prevention of cross contamination of infection between patients will be accomplished by rigorous cleaning and sterilization of the bronchoscope using procedures as outlined below.

1. Immediately after use, the suction channel is extensively rinsed with the remaining saline or tap water.
2. As soon as possible, the bronchoscope is cleaned thoroughly with an enzymatic soap solution.
 - a. Attach suction to suction outlet. Suction enzymatic solution through channel.
 - b. Brush the channel several times with a cleaning brush (the enzymatic soap should be suctioned through the channel while the brush is in the channel to ensure adequate cleaning action).
 - c. The main shaft of the bronchoscope is gently washed with a 4x4 and the enzymatic soap solution.
 - d. Rinse the main shaft and the suction channel with water.
 - e. Completely disassemble suction adapter. Using a brush and the enzymatic solution, clean the suction adapter and the top end of the suction channel.

- f. Dry the bronchoscope and suction adapter. Rinse mucus off the cleaning brushes.
- g. Completely immerse the bronchoscope, brushes, and suction adapters in the cold disinfectant solution (Cidex).
3. The scope must be soaked according to the cold disinfectant manufacturer's recommendations for disinfection.
4. Using a syringe with green bubble tubing, draw cold disinfectant through the suction channel during procedure.
5. After disinfection, the scope must be rinsed thoroughly with sterile water.
6. Attach a clean suction tubing to the bronchoscope and rinse suction channel thoroughly.
7. Draw 100cc of alcohol through bronchoscope.
8. Connect an airflowmeter to the wall outlet. Attach green bubble supply tubing to the flowmeter and the bronchoscope. Turn flowmeter on to 10 lpm. Blow air through scope 3-5 minutes.
9. Place cleaned/dry bronchoscope in protective wrap. (Rolls of protective wrap can be found in security cabinet.)
10. Place the bronchoscope in the security cabinet (located outside the endoscopy suite beside the door) for storage.

5.2.5 Blood specimens: The study subjects will have blood drawn a total of 21 times during the study. Blood will be drawn by venipuncture with no more than 20 ml taken at one time. This is less than 1% of the total blood volume for patients of this age.

5.2.6 Patient participation in concurrent studies: Study subjects will not be participating in concurrent protocols.

5.2.7 Facilities: Study patients will be evaluated initially in the cystic fibrosis clinic (Treatment Center 6), and the clinical research center (CRC). Spirometry will be performed in the pediatric pulmonary function laboratory (ASB-4). Fiberoptic bronchoscopy will be performed in the pediatric endoscopy suite located in Treatment Center 6. Throughout the admissions, patients will be isolated in a negative pressure room in the clinical research center (Room P1-16A). In this room, the air is filtered and exhausted to the outside. Full respiratory precautions will be observed, and anyone entering the room will wear a mask, a gown, gloves, and protective eye shields. When subjects leave the room for special procedures such as pulmonary function testing or fiberoptic bronchoscopy, they will wear fine particle respirators. It is anticipated that a second class A isolation room will be made available by the CRC for this study.

5.2.8 Special considerations

Radiation Safety: Participants in this protocol will receive AP and lateral chest x-rays 12 times in 5 months, with an exposure of 25mrem per pair of chest x-rays (AP 10mrem, lateral 15mrem). The total radiation dose from chest radiographs will therefore be 300mrem, which is increased compared to our routine clinical care that involves 25-50mrem per annum for 1-2 sets of AP and lateral chest radiographs. The annual public exposure limits recommended by the National Council for Radiation Protection are 100mrem per year for continuous or frequent exposure and 500mrem per year for infrequent exposure.

Dosimetry from Scans: The estimated dosimetry has been calculated for the scans that will be performed.

- a. Estimated Dosimetry: Tc-99m-pyrophosphate
Dosage and Route: 0.25 mCi by inhalation
Target organ absorbed dose:

Assumption 1: Uniform lung distribution

Whole body - approximately 0.003 mCi

Lungs - approximately 0.050 mCi

Assumption 2: All aerosol in trachea and larger bronchi

Whole body - approximately 0.003 mCi

Bronchial epithelium - approximately ?

- b. Dosimetry: Thin section CT of the lung
(1.5 mm thick sections at 10 cm intervals)
Six sections to be acquired

Dosimetry: Tc-99m macroaggregated albumin (MAA)

	<u>rad/2 mCi</u>
Whole body	0.024
Lungs	0.40
Liver	0.065
Spleen	0.034
Kidneys	0.022
Bladder wall	0.24 *
Testes	0.013 *
Ovaries	0.020 *

* 4.8 hr void

Source: Package insert, AN-MAA, Bedford MA: CB US, Inc, 1988.

The patients participating in this study will be adults aged 18 years or more, with mild to moderate cystic fibrosis lung disease. The median survival age of these patients is currently 29 years. The proposed study is a phase I study, so there will be no long-term benefits to the patients involved in the experimental protocol. However, the study could have considerable benefit to cystic fibrosis patients in general and to the study participants, in the long term. The information gained by the study will provide critical information for the development of gene therapy and a possible cure for cystic fibrosis pulmonary disease. We therefore feel that the radiation dose is safe and acceptable for the study participants.

Social Isolation: The patients in this protocol will be in respiratory isolation for approximately 4 weeks. During this time they will have a television and VCR available for entertainment. They will be allowed visitation by family members, who will be required to follow the respiratory isolation procedures. We will provide the patients with an exercise bicycle to allow

them an opportunity to exercise and to relieve stress and boredom. We will also provide them with any books, tapes, or magazines that they might wish to read or listen to.

5.3 Potential discomfort and risk

Venipuncture: Drawing of blood for these studies involves a small prick of the skin. Sometimes patients become faint, and occasionally there is bruising at the site of venipuncture. The risk for serious complication is small.

Pulmonary function testing: There are no significant risks associated with pulmonary function testing.

Perfusion scans, CT scans, and mucociliary clearance:

Risk: Allergy and toxicity are not known to occur. Each set of tests (aerosol, SPECT perfusion, and thin section CT, with a reduced number of sections) will be equal in dose to the average absorbed dose to lung in the standard thin-section CT protocol.

Discomfort: There will be the minor discomforts of lying on a hard imaging table (twice) and inhaling an aerosol by mask or mouthpiece. There will be a single venipuncture (for injection of Tc-99m-MAA for lung perfusion SPECT).

Fiberoptic bronchoscopy: The risks of bronchoscopy and bronchoalveolar lavage are those associated with hypoxemia due to the bronchoscopy itself, especially in patients with advanced lung disease. In our experience, with necessary precautions, if the oxygen saturation is greater than 90% at the time of bronchoscopy, then desaturation will not be a significant problem. All patients will be monitored with oximetry during the bronchoscopy, and if they have saturations less than 90%, supplemental oxygen will be administered.

Bleeding, pneumothorax, and respiratory arrest are potential complications of bronchoscopy and lavage, but they have not been encountered to date in cystic fibrosis patients receiving only fiberoptic bronchoscopy and bronchoalveolar lavage

It is possible that bleeding will develop following bronchial biopsy. This is a well established clinical procedure, and the risk is relatively low. The instance of moderate or severe bleeding when coagulation is normal is less than 1%. Serious bleeding is very uncommon and, when it occurs, usually affects patients affected by a coagulation disorder.

Vector related risk: There are several safety issues related to the use of a replication-deficient recombinant adenovirus to correct the respiratory aspects of cystic fibrosis. These risks include:

1. Will the adenovirus become infectious?
2. Will the vector express viral genes?
3. Could the CFTR gene be transferred to the germ line?
4. Do anti-adenoviral antibodies develop?
5. Will the patients become immune to the adenovirus vector?
6. Does administration of the recombinant adenovirus cause infection of the lung with possible inflammation?
7. Could the vector transform respiratory cells and cause malignancy?
8. Could over-expression of CFTR lead to deleterious changes in the recipient's lungs?

These points are addressed in detail in section 4.3. *In vivo* Use of a Replication Deficient, Recombinant Adenoviral Vector to Transduce Epithelial Cells of The Human Respiratory Tract.

Based on a risk/benefit judgment and on knowledge of the condition being studied, we would recommend that the protocol be classified as more than minimal risk but without direct benefit to participants.

5.4 Period of time for study completion: Patients will be initially treated for two weeks of intravenous antibiotics prior to initiation of the vector delivery. Participants will be treated twice and nursed in isolation until the viral surveillance cultures show that the adenovirus has been eliminated. It is anticipated that this will require 2 weeks of hospitalization with each treatment. Then they will be followed until 56 days (8 weeks) after therapy. Therefore, the total duration will be approximately 20 weeks per patient. We anticipate that it will take approximately 3 years to complete this study.

5.5 Funding: The cost of this study will be supported by grants from the Cystic Fibrosis Foundation and the National Institutes of Health. There will be no additional charges made to the patients or their families.

5.6 Payment for studies: There will be no compensation for participation in this study.

5.7 Method to be used in procuring consent of subjects: The patients participating in this protocol will receive a full oral explanation of the goals and the methods of this study. Fully informed consent will be obtained in every case. A copy of the consent form is attached.

5.8 Permission of the patient's attending physician: All patients to be enrolled in this study are followed at Children's Hospital Medical Center. Their attending physician(s) are members of the Division of Pulmonary Medicine, and they will be asked for permission to enroll their patients.

SECTION 7.

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Children's Hospital Medical Center

Consent to Participate in a Research Study

A Phase I Study of Gene Therapy of Cystic Fibrosis Utilizing a Replication
Deficient Recombinant Adenovirus Construct to Deliver the Cystic Fibrosis Transport
Regulator Gene to the Airways

Before agreeing that I will participate in this study, it is important that I read and understand the following explanation. It describes in words that can be understood by a lay person the purpose, the procedures, the benefits, risks, and discomforts of the study, and the precautions that will be taken. It also describes the alternatives available and the right to withdraw from the study at any time. It is important to understand that no guarantee or assurance can be made as to the results of this study. It is also understood that refusal to participate will not influence the availability of standard medical treatment.

I, _____ of _____
Patient Street
Address

_____, _____, _____, voluntarily
consent
City State Zip

for myself _____, _____ to participate
Name Age

in a research study, the purpose of which is to evaluate the safety and feasibility of using gene therapy in cystic fibrosis with a replication deficient adenovirus to deliver the normal cystic fibrosis transmembrane conductance regulator (CFTR) gene to the upper and lower airways. I was selected to participate in this study because I am an adult with cystic fibrosis and mild to moderate lung disease. The cystic fibrosis transmembrane conductance regulator is the protein that is absent or deficient in people affected by cystic fibrosis. CFTR can now be manufactured in the laboratory as the gene for cystic fibrosis was identified in 1989. Adenovirus is a cold virus which has been altered in the laboratory so that it will not reproduce itself in my body and so that it can carry the normal version of the CFTR gene into the cells of my lungs and nose.

The objective of this study is to determine whether the adenovirus-CFTR construct can be given into my nose and lower airways with incorporation in my cells resulting in manufacture of the normal CFTR by my body. This study will establish whether manufacture of the CFTR protein by these cells corrects the abnormal electrical voltages in the nose and airways of CF patients. This study will also evaluate the safety of this

form of gene therapy in patients with cystic fibrosis and determine whether my body forms antibodies to the adenovirus-CFTR construct.

January 20, 1993

If I decide to participate, I will be seen first for some initial studies in the clinical research center. These studies would decide whether or not I am eligible for this study. I will have a medical history, physical examination, EKG, chest x-ray, scans of my lungs, breathing tests, oxygen saturation measurement, brushings of cells from my nose, a bronchoscopy, cultures, blood tests, a urine test, a sputum culture, a genetic analysis for cystic fibrosis, a repeat sweat test, and a test for AIDS. If I am female, I will also receive a pregnancy test.

After this initial evaluation period, I will be admitted to the clinical research center for about 4½ weeks. I will be in isolation, but I will be allowed visitors who must follow isolation precautions. I will receive 2 weeks of intravenous antibiotics with aerosol therapy, chest percussion, postural drainage, and intensive nutritional support to prepare me for the study. After this 2-week cleanout, I will have my history and physical, chest x-ray, lung scans, oxygen saturation, pulmonary function test, viral cultures of throat and stool, blood tests, and urinalysis repeated. At that time the electrical voltage in my nose will be measured with a sensitive volt meter.

On the following day, I will receive a small dose of the adenovirus CFTR construct into one nostril. This will be performed with local anesthetic to my nose. The doctors will examine the inside of my nose with a fine telescope and select an area where the virus will be given.

One day later the electrical voltages within my nose will be measured again. I will also have a history and physical, culture for viruses, blood tests, and a urinalysis.

On the following day (day 0) I will receive the construct into my lung. I will have a history and physical repeated. I will receive intravenous sedation and the inside of my nose and the upper airway will be anesthetized with lidocaine. Cells will be obtained from inside my nose by brushing, and a small biopsy will be taken. The bronchoscopy will then be repeated. Washings will be taken from two lobes within my lungs, the airways will be brushed with a fine brush to obtain cells, and small biopsies will be taken. I will then receive a dose of the adenovirus-CFTR construct directly into one selected lobe. This lobe will be selected on the basis of it being a typical lobe by chest x-ray and lung scan. The equivalent lobe on the other side will be used for comparison. At the end of the procedure, the doctors will "paint" the inside of my windpipe with the viruses mentioned. Blood tests and a urinalysis will also be performed this day.

On day 3 (3 days after lung treatment) the bronchoscopy will be performed again as before, and the voltages will be measured within my windpipe. After measuring the voltages, the doctors will be able to anesthetize my windpipe and lower airways. Washings from the lung, brushings, and biopsies will be obtained. Chest x-ray, oxygen saturation, pulmonary function tests, blood tests, and a urine test will also be performed.

One week later (day 7) and two weeks later (day 14) the history and physical will be repeated, the chest x-rays will be repeated together with oxygen saturation tests and pulmonary function tests. The lung scans will be repeated 7 days later. Virus cultures will be obtained and blood tests will be collected, as well as urine tests. If I am well and if the cultures from my throat swabs and stool show that no adenovirus is being excreted from my body at day 14, I will be discharged. In the unlikely event that I am still excreting the adenovirus construct, I will be asked to remain in isolation in the clinical research center until the virus has been eliminated.

I will then be followed in the outpatient clinic, and every week I will attend for a history and physical, oxygen saturation tests, pulmonary function tests, adenovirus cultures, blood tests, and urine tests. The chest x-ray will be performed again on day 28. During this period of time when I am at home following gene therapy, I will be asked to avoid contact with people with known infections and young children, and to avoid crowded places such as movie theaters and shopping malls.

On day 55 I will be readmitted to the clinical research center and will have a history and physical, chest x-ray, lung scans, oxygen saturation tests, pulmonary function tests, virus cultures, blood tests, urine tests, sputum culture, and if I am female, a repeat pregnancy test.

On the following day (day 56) the voltages in my nose will be measured again. After anesthetizing the nose with lidocaine, the nose will be brushed and small biopsies taken. I will then have my upper airways anesthetized with lidocaine, and with intravenous sedation I will have a repeat bronchoscopy with bronchial washings, brushings, and small biopsies. After collecting tissues the doctors will administer a dose of the adenovirus-CFTR construct directly into one selected lobe, which will be the same lobe that was treated before. At the end of the procedure, the doctors will "paint" the inside of my windpipe with the virus suspension again. Adenovirus cultures will be collected prior to bronchoscopy and administration of the construct. Blood tests and a urine test will be performed.

On day 59 a history and physical, chest x-ray, oxygen saturation tests, pulmonary function tests, adenovirus culture from pharyngeal swabs and stool, blood tests, and urinalysis will be performed. The bronchoscopy will be repeated with intravenous sedation as before. The voltage inside my windpipe will be measured. Bronchial washings, brushings, and small biopsies will be obtained.

On day 63 I will have a history and physical, chest x-ray, lung scans, oxygen saturation tests, pulmonary function tests, adenovirus cultures from pharyngeal swabs and stool, blood tests, and urinalysis.

One week later (day 70) I will have the same tests, except for lung scans, which will not be performed. If my culture for the recombinant adenovirus is negative, I will come out of isolation and be discharged again. I will then return at weekly intervals for a history

and physical, oxygen saturation tests, pulmonary function tests, adenovirus cultures, blood tests, and urine tests. Chest x-rays will be performed on days 84 and 112. Lung scans will be repeated on day 112.

On days 84 and 112 I will have repeat measurements of the voltage inside my nose, nasal brushings and biopsies, and repeat bronchoscopies under intravenous sedation.

BENEFITS

If I participate in this study, I will be participating in one of the first studies of gene therapy for cystic fibrosis in human beings. These studies are being performed to establish whether it is safe to give this treatment and whether the treatment is feasible. It is unlikely that there is direct benefit to me by participating in this study, except for the benefit of very close medical attention and a free 2-week course of intravenous antibiotics. I understand that if gene therapy in cystic fibrosis is successful, there will be eventually be benefit to all cystic fibrosis patients including me.

RISKS, DISCOMFORTS, AND PRECAUTIONS

Participation in this study poses the following risks and discomforts for me:

- a. Pain, bruising, and potential infection at the site of blood sampling. During the 6 weeks of the study, blood will be drawn 21 times, but no more than 20cc (4 tsp) will be drawn at one time.
- b. Pulmonary Function Testing: There are no significant risks associated with pulmonary function testing, which requires me to blow into a cardboard mouthpiece.
- c. Lung Scans: I will have the minor discomforts of lying on a hard surface while being scanned and breathing an aerosol. I will also have an injection of technetium-labelled protein for each perfusion scan. There have been no adverse reactions to this protein, but allergic reactions are a possibility.
- d. Fiberoptic Bronchoscopy: There are several risks associated with the bronchoscopy:
 1. Coughing: Spraying the back of the throat and the airways with lidocaine often causes coughing until the numbness takes effect. Coughing sometimes occurs when the bronchoscope is in the airway.

2. Low Oxygen Level: Sometimes the presence of the bronchoscope in the airway causes the oxygen level in the blood to drop. This will be monitored during the procedure, and I will be given oxygen to breathe if the saturation drops below 90%. If the saturation remains below 90%, the bronchoscope will be removed from the airway.

3. Bleeding: Taking small pieces of tissue from the airways and brushing the lungs with a fine brush can cause bleeding. The bleeding is usually minor and stops quickly. Very occasionally the bleeding is more severe. The bronchoscope can be used to temporarily plug the bleeding airway and to encourage the bleeding to stop or a dilute epinephrine solution can be injected through the bronchoscope. Rarely, less than 1% of the time, the bleeding is more severe. In such cases, it is unusual for a blood transfusion to be used. Severe bleeding of this type has never occurred in our experience with patients who have normal blood clotting and who don't have cancer.
4. Pneumothorax: When pieces of tissue are removed from the airway, sometimes an air leak develops which can result in a collapsed lung. The leak allows the lung to collapse either completely or partially. If the lung were to collapse completely, a tube would need to be inserted through an incision between the ribs to remove the air. If used, the tube stays in place for 3-5 days until the air leak stops. It is rare for healthy patients receiving bronchoscopy with biopsy to develop a pneumothorax, and the risk of this is less than 1%.
5. Respiratory Arrest: Cessation of breathing during the bronchoscopy could occur as a response to the intravenous sedation that I will receive. This has not been encountered to date in cystic fibrosis patients undergoing fiberoptic bronchoscopy with the procedure that will be used. Heart rate, respiratory rate, and blood pressure will be closely monitored throughout the procedure. Emergency equipment is immediately available if needed.
6. Fever: After washings of the lower airway, it is possible for fever to develop within the next 24 hours. I have about a 1 in 20 chance of developing this problem.
7. Deterioration in Pulmonary Function: Repeated bronchoscopy could lead to a deterioration in pulmonary function in cystic fibrosis. It is not standard care for CF patients to receive 18 bronchoscopies in 4 months, and the passing of the instruments through the airways could cause inflammation, irritation, or an increase in infection. However, repeated bronchoscopies have been performed in relationship to clinical research in CF, and complications have been rare in skilled hands.

e. Risks Related to the Virus:

It is possible that the virus could cause the following complications:

1. Overproduction of the cystic fibrosis protein. It is possible that the gene therapy will cause too much CFTR to be made in the airway cells. Animal research has suggested that this is not going to be a hazard in humans. Tests in animals have shown that increasing the amount of CFTR protein made by many times does not cause any problems. Whether this is true in humans is not known.
2. Multiplication of the virus. The virus that I will receive into my airways is being modified so that it cannot reproduce itself. It is possible that something will alter the virus in my body so that it becomes infectious again. It is thought by the doctors that this is a very remote possibility, but the effects of this happening are unknown. If the virus were to become infectious, my body would probably start to kill the virus after a short time, just as when people have colds.
3. Damage to the airway cells. The virus could injure the cells in my nose and lower airways. The research performed so far with animal and human cells in the laboratory have not shown that this happens. However, if the airway cells were damaged, it is possible that the lining of my nose, or the lobe that is treated, could be damaged by the virus either temporarily or permanently.
4. Environmental spread of the virus. It is possible that the virus will become infectious and leave my body and spread into the environment. However, the virus has been modified in the lab so that this should not occur. The adenovirus probably would not behave any differently to other types of adenovirus that are already present in the environment. If my body continues to excrete the virus at the end of the study, I will be asked to stay in the hospital until my cultures become negative.
5. Malignancy. The cold virus being used in these studies is not one which causes cancer. Other types of virus can damage normal genes when they insert into the chromosomes. Because the adenovirus does not usually insert into genes on the chromosomes, it is very unlikely that the virus could cause cancer.
6. Modification of the sperm or eggs. If the virus spread through my body, it is possible that the cystic fibrosis gene could be inserted into the body's eggs or sperm. I have been informed by the doctors that I can only participate in the study if I am sterile, to avoid the chance that I could pass genetic changes in my body's eggs or sperm to children. It is thought by the doctors that this is a very remote

possibility.

- f. Radiation Safety: I will receive 12 pairs of chest x-rays during the 4½ months that I will be participating in this study. Each pair of x-rays, a front and a side view, give me a dose of 25mrem, so the total dose will be 300mrem. I will also receive some radiation exposure from the lung scans. The chances of developing cancer from this amount of radiation are very small. This dose of x-rays is within the official radiation exposure limits for the public, so long as my exposure to x-rays is infrequent.

Although the above potential risks are rarely of clinical significance, my condition will be monitored throughout the study, and every precaution consistent with the best medical care will be taken.

I have been informed that patients who participate in Phase I trials are sometimes excluded from future experimental therapies, although this is not the intention of my doctors. I understand that participation in this study could exclude me from later (Phase II and Phase III) trials until gene therapy is commercially available.

I understand that if I believe that I have been injured as a result of participation in biomedical or behavioral research I am to contact Dr. Robert Wilmott or the Director of Social Services to discuss my concerns. Children's Hospital Medical Center follows a policy of making all decisions concerning compensation and/or medical treatment for physical injuries occurring during or caused by participation in biomedical or behavioral research on an individual basis.

ALTERNATIVES

An alternative treatment for me is standard cystic fibrosis care (chest physiotherapy, antibiotics, nutritional support, pancreatic enzymes, and vitamins) without gene therapy.

CONFIDENTIALITY OF RECORDS

All information gathered in this study will be kept confidential to the extent provided by federal, state, and local law. Records relating to my participation in this study will be protected against release to unauthorized people. In addition to the members of the health care staff who usually have access to my file, the records may be reviewed by Food and Drug Administration and Genetic Therapy, Inc.

AVAILABILITY OF INFORMATION

If I have any questions regarding this study, my rights as a research subject, or I think

that I have suffered an adverse reaction, I may talk to Robert W. Wilmott, M.D., Director, Division of Pulmonary Medicine, Children's Hospital Medical Center, Elland and Bethesda Avenues, Cincinnati, Ohio, 45229, telephone number (513) 559-6771, or Jeffrey A. Whitsett, M.D., Director, Pulmonary Biology, Children's Hospital Medical Center, Elland and Bethesda Avenues, Cincinnati, Ohio, 45229, telephone number (513) 559-7665.

THE RIGHT TO WITHDRAW

I am free to withdraw myself from this investigation at any time without jeopardizing future care at the hospital. Standard therapy will remain available if I choose to withdraw from the study.

WITNESS AND SIGNATURES

My signature on this consent form indicates that I have read and understand the purpose, procedures, risks, and benefits of participation in this study. I have been given the opportunity to ask questions and my decision to participate is based on the information provided. A copy of this form will be given to me.

Patient's signature indicating consent

or

☐

check box if
verbal assent
obtained by
investigator

Witness as to Voluntary Signature

Date

INVESTIGATOR'S STATEMENT

I have fully explained to _____ (patient) the nature and purpose of the above described procedure and the risk involved in its performance. I have answered and will answer all questions to the best of my ability. I will inform the participant of any changes in the procedure, or risk or benefits if any should occur during or after the course of this study.

Signature of Investigator

Date

BASIC ELEMENTS OF INFORMED CONSENT

The DHS and FDA Policies on Protection of Human Subjects list the basic elements of informed consent as follows:

1. A statement that the study involves research, an explanation of the purposes of the research and the expected duration of the subject's participation, a description of the procedures to be followed, and identification of any experimental procedures.
2. A description of reasonably foreseeable risks and discomforts.
3. A description of any benefits to the subject or others that can reasonably be expected from the research.
4. A disclosure of appropriate alternative procedures or courses that might be advantageous to the subject.
5. A statement describing the extent, if any, to which confidentiality of records identifying the subject will be maintained.
6. An explanation as to whether any compensation and medical treatment is available if injury occurs.
7. An explanation of whom to contact for answers to research-related questions and whom to contact in the event of a research-related injury.
8. A statement that participation is voluntary, that refusal to participate will not involve loss of benefits, and that subjects may discontinue participation at any time without loss of benefits.

This study has been reviewed and approved by the Institutional Review Board of the Children's Hospital Medical Center (Chairman's office telephone number, 559-8039).

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME Robert W. Wilmott, M.D.	POSITION TITLE Director, Pulmonary Medicine		
EDUCATION (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)			
INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
University College, London, England	BSc	1970	Anatomy/Preclin
University College, London, England	MB BS	1973	Medicine
London University, London, England	MD	1984	Research Thesis: Allergy and the Lung Disease of CF

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

Employment - Professional Experience:

6/76-11/76 Senior House Officer, Neonatal Medicine, University College Hospital, London
 1/77-6/77 Senior House Officer, The Hospital for Sick Children, London
 1977-1978 Instructor in Pediatrics, University of Pennsylvania
 1977-1978 Assistant Chief Resident, The Children's Hospital of Philadelphia
 1978-1979 Registrar in Pediatrics, The Hospital for Sick Children, London
 1979-1981 Pulmonology and Cystic Fibrosis Research Fellow, Honorary Senior Registrar, Hospital for Sick Children, London, & Institute of Child Health, London
 1980-1981 Chairman, Junior Staff Committee, The Hospital for Sick Children, London
 1981-1986 Assistant Professor of Pediatrics, University of Pennsylvania
 1982-1986 Clinical Director, Pulmonology Section; Co-Director, Cystic Fibrosis Center; Director, Pulmonary Function Laboratory, The Children's Hospital of Philadelphia
 1983-1985 Graduate Education Committee, Children's Hospital of Philadelphia
 1983-1986 Medical Devices Committee, Children's Hospital of Philadelphia
 1985-1986 Human Subjects Committee, Children's Hospital of Philadelphia
 1986-1989 Director, Division of Pulmonary Medicine, Children's Hospital of Michigan
 1986-1989 Medical Director, Respiratory Therapy Department, Children's Hospital of Michigan
 1986-1989 Associate Professor of Pediatrics, Wayne State University
 1987-1989 Associate in Immunology, Department of Immunology and Microbiology, Wayne State University School of Medicine
 1989-Pres. Associate Professor, Department of Pediatrics, University of Cincinnati College of Medicine
 1989-Pres. Director, Division of Pulmonary Medicine, Children's Hospital Medical Center

Major Professional Societies:

The Neonatal Society, London
 The British Pediatric Association
 The Reticuloendothelial Society
 The Society for Experimental Biology and Medicine
 Member, Michigan Thoracic Society
 Society
 Member, The American Association of Immunologists

Fellow, The Royal Society of Medicine, London
 American Thoracic Society
 The American Society for Microbiology
 Fellow, The American Academy of Pediatrics
 Member, The Society for Pediatric Research
 Member, European Pediatric Respiratory
 Fellow, The American College of Chest Physicians

Honors and Awards:

- 1969-1970 Full Scholarship, B.Sc., Medical Research Council, England
 1970 The Laurels Award, University of London Union (for Administrative Services)
 1972 Honorary Life Membership, University of London Union
 1973 The Magrath Scholarship in Medicine, University College Hospital
 1973 The Belasco Medal, University College Hospital, London (award for medicine in final MB examination)
 1987 Award of appreciation, The Metropolitan Detroit Cystic Fibrosis Foundation

Selected Bibliography:

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2. Pitcher-Wilmott RW, Shutack JG, Fox WW: Decreased Lung Volume after Nasogastric Bolus Feeding of Neonates Recovering from Respiratory Disease. *Journal of Pediatrics* 1979; 95:119-121.
3. Pitcher-Wilmott RW, Hindocha P, and Wood CBS: The Placental Transfer of IgG Subclasses in the Human Pregnancy. *Clinical Exp. Immunol* 1980; 41:303-308.
4. Pitcher-Wilmott RW, Levinsky RJ, Gordon I, Turner MW, Matthew DJ: Pseudomonas Infection, Allergy and Cystic Fibrosis. *Archives of Disease in Childhood* 1982; 57:582-586.
5. Pitcher-Wilmott RW, Levinsky RJ, and Matthew DS: Circulating soluble immune complexes containing pseudomonas antigens in children with cystic fibrosis. *Archives of Disease in Childhood* 1982; 57:577-581.
6. Wilmott RW, Tyson SL, Dinwiddie R, and Matthew DJ: Survival rates in cystic fibrosis. *Archives of Disease in Childhood* 1983; 58:835-836.
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8. Ruddy RM, Kolski G, Scarpa N, Wilmott RW: Repeated aerosolized metaproterenol compared to repeated subcutaneous epinephrine injections in acute childhood asthma. *Pediatric Pulmonology* 1986; 2:230-236.
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10. Hoyt RW, Scarpa N, Wilmott RW, Cohen A, Schwartz E: Pulmonary function abnormalities in patients homozygous for β -thalassemia. *Journal of Pediatrics* 1986; 109:452-455.
11. D'Andrea AD, Bayever E, Haines KM, Wilmott RW, Polin RA, August CA, and Douglas SD: Serial evaluation of lymphocyte function in bone marrow-grafted patients. *Diagnostic and Clinical Immunology* 1986; 4:281-287.
12. Wilmott RW, Harris MC, Haines KM, and Douglas SD. Interleukin-1 Activity from Human Cord Blood Monocytes. *Diagnostic and Clinical Immunology* 1987; 5:201-4.
13. Smith AL, Redding G, Doershuk C, Goldmann D, Gore E, Hilman B, Marks M, Moss R, Ramsey B, Rubio T, Schwartz R, Thomassen MJ, Williams-Warren J, Weber A, Wilmott RW, Wilson HD, and Yogev R. Sputum changes with therapy in cystic fibrosis. *Journal of Pediatrics* 1988; 112:547-54.
14. Garty BZ, Levinson AI, Danon YL, Wilmott RW, and Douglas, SD. Lymphocyte subpopulations in children with abnormal lymphatic circulation. *Journal Allergy Clinical Immunology*; 1989; 84:515-20.
15. Wilmott RW, Kassab JT, Kilian PL, Benjamin WR, Douglas SD, and Wood RE. Increased levels of interleukin-1 in bronchoalveolar washings from children with bacterial pulmonary infections. *American Review of Respiratory Disease*; 1990 (in press).
16. Garty BZ, Athreya, BH, Wilmott RW, Scarpa N, Doughty R, Douglas S: Pulmonary functions in children with progressive systemic sclerosis. *Pediatrics* 1991; 88(6):1161-1167.
17. Nasr SZ, Amato P, Wilmott RW: Normal values for lung diffusing capacity in children. *Pediatric Pulmonology* 1991; 10:267-272.
18. Kaplan EB, Wodell, RA, Wilmott RW, Leifer B, Lesser ML, August CS: Chronic graft-versus-host disease and pulmonary function. *Pediatric Pulmonology* 1992; 14(3):141-148.

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME Jeffrey A. Whitsett, M.D.	POSITION TITLE Professor of Pediatrics		
EDUCATION (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)			
INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
Colgate University, New York	B.A.	1969	Chemistry
Columbia University, New York	M.D.	1973	Medicine
Mt. Sinai Hospital, New York		1973-76	Internship-Residency
Children's Hospital, Cincinnati, OH		1976-78	Neonatal Fellowship

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

Employment:

1985-present Professor of Pediatrics, and Pharmacology and Cell Biophysics, University of Cincinnati; Director, Pulmonary Biology, Children's Hospital Medical Center

Publications: (Selected from 124 peer reviewed manuscripts)

1. Whitsett, J.A., Pilot, T., Clark, J. and Weaver, T.E.: Induction of surfactant protein in fetal lung: effects of cAMP and dexamethasone on SAP-35 RNA and synthesis. J. Biol. Chem. 262:5256-5261, 1987.
2. Glasser, S.W., Korfhagen, T.R., Weaver, T., Pilot-Matias, T., Fox, J.L. and Whitsett, J.A.: cDNA and deduced amino acid sequence of human pulmonary surfactant-associated proteolipid SPL(Phe). Proc. Natl. Acad. Sci. USA 84:4007-4011, 1987.
3. Whitsett, J.A., Weaver, T.E., Lieberman, M.A., Clark, J.C. and Daugherty, C.: Differential effects of epidermal growth factor and transforming growth factor- β on synthesis of Mr=35,000 surfactant-associated protein in fetal lung. J. Biol. Chem. 262:7908-7913, 1987.
4. Whitsett, J.A., Weaver, T.E., Clark, J.C., Sawtell, N., Glasser, S.W., Korfhagen, T.R. and Hull, W.M.: Glucocorticoid enhances surfactant proteolipid Phe and pVal synthesis and RNA in fetal lung. J. Biol. Chem. 262:15618-15623, 1987.
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10. O'Reilly, M.A., Weaver, T.E., Pilot-Matias, T.J., Sarin, V.K., Gazdar, A.F. and Whitsett, J.A.: In vitro translation, post-translational processing and secretion of pulmonary surfactant protein B precursors. Biochim. Biophys. Acta 1011:140-148, 1989.

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12. O'Reilly, M.A., Gazdar, A.F., Clark, J.C., Pilot-Matias, T.J., Wert, S.E., Hull, W.M. and Whitsett, J.A.: Glucocorticoids regulate surfactant protein synthesis in a pulmonary adenocarcinoma cell line. Am. J. Physiol. 257 (Lung Cell Mol. Physiol. 1):L385-L392, 1989.
13. Sarin, V.K., Gupta, S., Leung, T.K., Taylor, V., Ohning, B.L., Whitsett, J.A. and Fox, J.L.: Biophysical and biological activity of a synthetic 8.7 kDa hydrophobic pulmonary surfactant protein SP-B. Proc. Natl. Acad. Sci. USA 87:2633-2637, 1990.
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15. Korfhagen, T.R., Glasser, S.W., Wert, S.E., Bruno, M.D., Daugherty, C.C., McNeish, J.D., Stock, J.L., Potter, S.S. and Whitsett, J.A.: Cis-acting sequences from a human surfactant protein gene confer pulmonary-specific gene expression in transgenic mice. Proc. Natl. Acad. Sci. USA 87:6122-6126, 1990.
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23. Glasser, S.W., Korfhagen, T.R., Wert, S.E., Bruno, M.D., McWilliams, K.M., Vorbroker, D.K. and Whitsett, J.A.: Genetic element from human surfactant protein SP-C gene confers bronchiolar-alveolar cell specificity in transgenic mice. Am. J. Physiol.: Lung Cell Mol. Physiol. 261:L349-L356, 1991.
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CURRICULUM VITAE

NAME: Bruce Colston Trapnell

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19 Firstfield Road FAX: 301 590-2638
Gaithersburg, MD 20878

DATE & PLACE OF BIRTH: March 11, 1955; Portsmouth, Virginia, USA

CITIZEN: United States

LICENSURE: 1991, District of Columbia

BOARD CERTIFICATION: 1985, Diplomate, National Board of Medical Examiners
1987, Diplomate, American Board of Internal Medicine

EDUCATION AND CLINICAL TRAINING:

1973 - 1978 B.S., Biochemistry, University of Maryland, College Park, Maryland
1978 - 1981 M.S., Genetics, The George Washington University, Washington, DC
1980 - 1984 M.D., University of Maryland School of Medicine, Baltimore, Maryland
1984 - 1985 Intern in Medicine, The Ohio State University Hospitals, Columbus, Ohio
1985 - 1986 Junior Resident in Medicine, The Ohio State University Hospitals,
Columbus, Ohio. Earl Metz, M.D. Program Director.
1986 - 1987 Senior Resident in Medicine, The Ohio State University Hospitals,
Columbus, Ohio. Earl Metz, M.D. Program Director.
1987 - 1989 Clinical Fellow in Pulmonary Medicine, National Heart, Lung, and Blood
Institute, Clinical Center, National Institutes of Health, Bethesda,
Maryland. Richard Hubbard, M.D. Chief of Service.

PROFESSIONAL EXPERIENCE:

1978 Biological Laboratory Technician, Section on Pulmonary Biochemistry, National
Heart, Lung And Blood Institute, National Institutes of Health, Bethesda
Maryland

1978 - 1984 Chemist, Pulmonary Branch, National Heart, Lung and Blood Institute, National
Institutes of Health, Bethesda, Maryland

1984 - 1987 Housestaff Instructor, Department of Medicine, The Ohio State University
Hospitals, Columbus, Ohio

1987 - 1991 Senior Staff Fellow, Pulmonary Branch, National Heart, Lung, and Blood
Institute, National Institutes of Health, Bethesda, Maryland

1991 - 1992 Senior Investigator (NIH Special Volunteer), Pulmonary Branch, National
Heart, Lung, and Blood Institute, National Institutes of Health,
Bethesda, Maryland

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HUMAN GENE TRANSFER PROTOCOL

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III.C. One-page abstracts (scientific and non-technical)

Scientific

Cystic fibrosis (CF) is an autosomal recessive disease that reflects mutations in the CFTR gene. Multiple mutations in this gene have been detected that lead to a protein (CFTR) that is abnormally metabolized, dysfunctional, or both. The full spectrum of the activities of the gene product have not been defined, but it is clear that CFTR can act as a cAMP-regulated Cl^- channel. This type of defect is consistent with the physiologic characterization of CF epithelia, which has revealed abnormalities in salt and water transport. In the lung, abnormalities in epithelial salt and water metabolism lead to abnormal mucociliary clearance. This defect in clearance represents a major failure of lung defense and leads ultimately to infection of the lung with *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and other bacterial organisms. The chronic inflammatory response to this persistent intraluminal bacterial infection leads to protease-induced destruction of airway walls and finally, lung failure. More than 95% of CF patients die of lung disease.

The clinical therapy of CF lung disease is limited to agents designed to promote clearance of secretions from the lung and antibiotics to treat the chronic bacterial infection. Recent laboratory demonstrations that introduction of the normal CFTR cDNA into CF cells corrects the ion transport defects of these cells has led to the hypothesis that gene therapy in the lung can be an effective, novel mode of therapy for this lung disease. The classic gene transfer vectors, e.g., retroviruses, appear to be not well suited for therapy of lung disease because of the low proliferation rate of airway epithelia *in vivo*. Recently, adenoviruses, which have a natural tropism for airway epithelia, have been genetically modified (E1-deleted) in an attempt to reduce potential toxicity of this virus and provide space for the CFTR cDNA. A series of *in vitro* studies have shown that this vector is highly efficient for transferring CFTR into airway epithelial cells in culture and correcting the CF defect. Further, studies in whole animals appear to indicate that this mode of gene transfer is associated with a low degree of toxicity.

The present study is a dose-effect study designed to test for the safety and efficacy of E1-deleted recombinant adenovirus containing the CFTR cDNA under a CMV- β actin promoter in CF nasal epithelia. The nasal epithelium was selected for study because it exhibits the epithelial defects characteristic of CF, allows the administration of a low volume of virus to an accessible area of the epithelium, and is highly suited for multiple studies designed to measure toxicity and efficacy. A single dose will be administered to the nasal turbinate region of CF patients, with doses ranging from 10^8 to 10^{11} pfu/ml. Three patients will be selected for each dose (10^8 , 3×10^9 , 10^{11}). Beginning 24 hr after vector instillation, assessment of the effects of virus on safety will be sought utilizing nasal lavage for measurement of inflammatory cells, mediator release, and viral shedding, and intermittently by biopsies designed to evaluate nasal epithelial morphology and intracellular viral replication. Efficacy will be tested by restoration of electrolyte transport as measurement by the *in vivo* PD technique (a non-invasive measurement). These studies will be completed by immunocytochemical studies designed to test for expression of CFTR in nasal epithelial cells. The goal is to define the safety and efficacy of the various doses as a function of time, with the ultimate goal of using

these studies to understand safety, efficacy, and potential dosing intervals pertinent to this novel mode of therapy.

Non-technical

Cystic fibrosis (CF) is a genetic disease that affects ~1 in 2,500 live births in the United States. The gene that is abnormal in CF produces problems particularly in the tissues that line various body cavities. Generally, these tissues, termed epithelia, secrete a combination of water and mucus to protect the surfaces of these tissues from toxic/environmental insults from the outside world. These fluids are abnormally secreted and cleared from the lungs of CF patients, which leads to a chronic cycle of bacterial infection and damage of airway walls. Ultimately, most deaths from CF (>95%) result from lung tissue destruction and pulmonary failure.

The current therapies for treating CF lung disease focus on promoting clearance of secretions from the lungs and antibiotics designed to control the lung infection. Whereas these therapies have been effective in prolonging the life-span of CF patients to a median age of ~25 years, they have reached their maximum benefit. A recent series of studies in the laboratory has shown that if the normal version of the CF gene is inserted into CF cells, a correction of the epithelial defect in these cells is readily detected. This type of experiment, in which a gene (the normal CF gene) is transferred to a defective (CF) cell, is a type of gene transfer and has raised the question of whether similar types of gene transfer may be utilized clinically for therapy of CF lung disease.

The adenovirus is a "cold" virus that has a natural tendency to infect many tissues of the nasal cavity and lung. This feature, plus the ready access of the lung tissues to delivery of this kind of virus via aerosol or direct liquid instillation, indicates that this virus is potentially useful for delivering the normal CF gene therapeutically to the CF lung. Consequently, this virus has been genetically engineered by recombinant DNA technology so that some of the potentially toxic components of this virus have been removed and the normal copy of the CF gene inserted. A series of studies in animals and in human tissues in laboratory culture have shown that this virus appears to be very effective in introducing the normal CF gene into affected tissues without any major detectable toxic effects. In the current study, we plan to test this virus vector in the nasal cavity of CF subjects. The major focus of the study is designed to test whether the administration of this virus to a very localized region of the nasal cavity is safe, and whether it has the capacity to restore the functions of the nasal lining tissue to normalcy. The nasal cavity was chosen for these initial studies because of the small volume of virus needed to perform the tests, the fact that the nasal lining tissue exhibits the same types of abnormalities as are expressed in the lung, and that testing the effects of the virus can be performed repetitively with relatively little discomfort to the patient. The long-range goal of these studies is to test the feasibility of this type of virus for gene therapy of CF lung disease and to develop information that will lead to the design of better viruses and better modes of delivery.

Gene Therapy for Cystic Fibrosis Using E1 Deleted Adenovirus:

A Phase I Trial in the Nasal Cavity

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II. BACKGROUND

II.A. Cystic Fibrosis

Cystic fibrosis (CF) is monogenetic disorder that presents as a multisystem disease (1-6). The first signs and symptoms typically occur in childhood, but nearly 3% of patients are diagnosed as adults. Due to improvements in therapy, more than 25% of patients reach adulthood and >9% live past age 30. The disease is characterized by chronic airways infection that ultimately leads to bronchiectasis and bronchiolectasis, exocrine pancreatic insufficiency, abnormal sweat gland function, and urogenital dysfunction.

II.A.1. Pathogenesis

II.A.1.a. Genetic Basis:

CF is an autosomal recessive disease resulting from mutations in the CF gene located on chromosome 7 (7-9). The prevalence of CF varies with the ethnic origin of a population. CF is detected in approximately 1 in 2,500 live births in the Caucasian population of North America and Northern Europe, 1 in 17,000 live births of African-Americans, and 1 in 90,000 live births of the Asian population of Hawaii. The most common mutation in the CF gene is a three-base pair deletion that results in absence of phenylalanine at amino acid position 508 (ΔF_{508}) of CF gene protein product, the CF transmembrane regulator (CFTR).

II.A.1.b. CFTR Protein:

The CFTR protein is a single polypeptide chain, containing 1480 amino acids that appears to function as a cyclic AMP-regulated Cl^- channel. The fully processed form of CFTR is found in the plasma membrane in normal epithelia (10,11). Biochemical studies indicate that the ΔF_{508} mutation leads to improper processing and intracellular degradation of the CFTR protein (12,13). Thus, absence of CFTR protein at appropriate cellular sites may be a part of the pathophysiology of CF. However, other mutations in the CF gene produce CFTR proteins that are fully processed but are nonfunctional at the appropriate cellular sites.

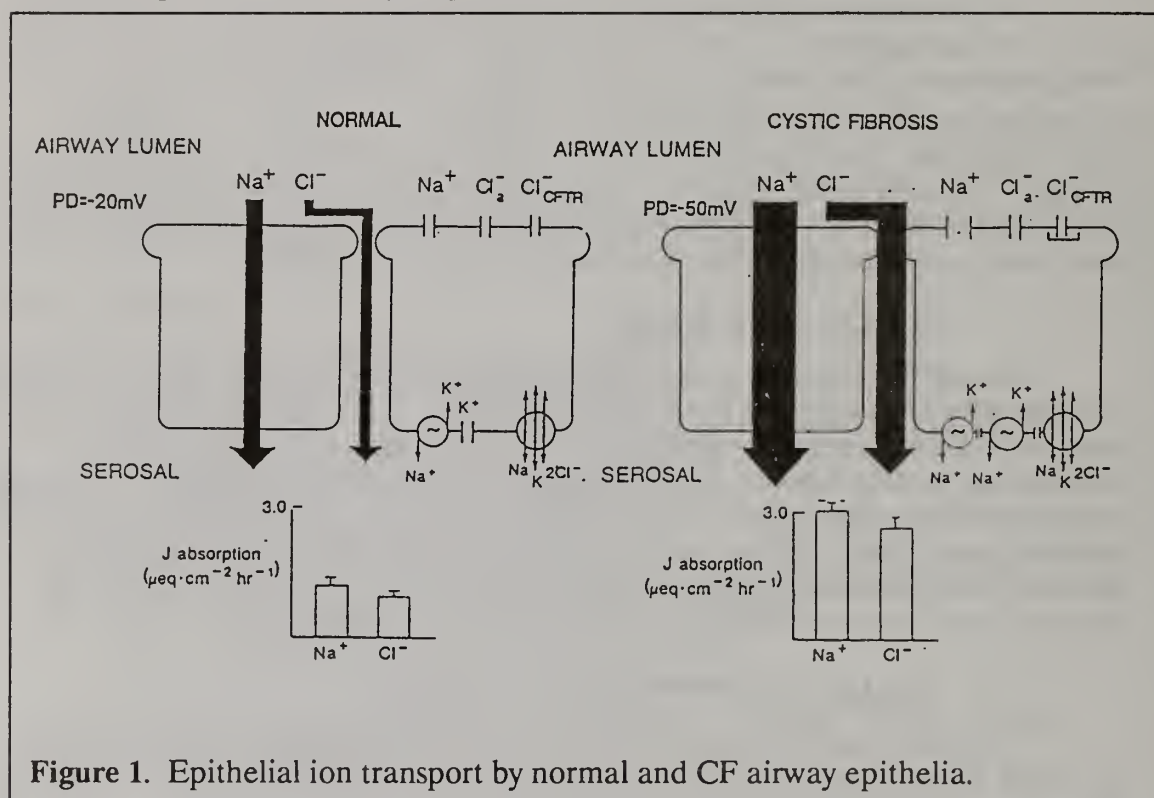
II.A.1.c. Epithelial Dysfunction-General:

The epithelia affected by CF exhibit in their native state different functions; i.e., some are volume absorbing (airways and intestinal epithelia), some are salt but not volume absorbing (sweat duct) whereas others are volume secretory (pancreas). Given this diverse array of native activities, it should not be surprising that CF produces very different effects on patterns of electrolyte and water transport (2,4,14). However, the unifying concept is that all affected tissues express abnormal cAMP-regulated Cl^- channel activity.

II.A.2. Organ-specific pathophysiology

II.A.2.a. Lung:

The diagnostic biophysical hallmark of CF is the raised transepithelial electric potential difference (PD) detected in airway epithelia (15). The transepithelial PD reflects components of both the rate of active ion transport and the resistance to ion flow of the superficial epithelium. CF airway epithelia exhibit both raised transport rates (Na^+) and decreased ion permeability (Cl^-) (16,17) (Figure 1). The Cl^- transport defect appears to be the result of abnormal regulation of Cl^- transport (17,18). CF epithelia do not respond to β -agonists or agonists that activate protein kinase C with Cl^- secretion as normal airway epithelia do (19,20). This failure to regulate cellular Cl^- transport is a direct consequence of mutations in the CFTR protein (shown by "cap" on Cl^- CFTR in Figure 1).



Raised Na^+ absorption is a feature of CF airway epithelia. Na^+ transport abnormalities in CF are not a widespread feature of the CF epithelial phenotype and appear confined to volume absorbing epithelia. The mechanisms for Na^+ hyperabsorption reflect in part an increase in apical cell membrane Na^+ channel activity. It is not yet known how the abnormal CF gene product, CFTR, produces the Na^+ transport defect.

The central hypothesis of CF airways pathophysiology has been that the abnormal Na^+ and Cl^- transport rates produce secretions that are dehydrated and poorly cleared. The unique predisposition of CF airways to chronic infection by *Staphylococcus aureus* and *Pseudomonas aeruginosa* raises the issue that other as yet

undefined abnormalities in airway surface liquids may also contribute to the failure of lung defense.

II.A.2.b. Gastrointestinal:

The gastrointestinal effects of CF are diverse (21). In the exocrine pancreas, it appears that the absence of the CFTR Cl^- channel in the apical membrane of pancreatic ductal epithelia impairs the activity of an apical membrane Cl^- - HCO_3^- exchanger to effect net secretion of bicarbonate and Na^+ (by a passive process) into the duct. The failure to secrete Na^+ - HCO_3^- and water leads to retention of enzymes in the pancreas and ultimately destruction of virtually all pancreatic tissue. In CF intestinal epithelium, a lack of Cl^- and water secretion impairs the ability to flush the secreted mucins and other macromolecules from intestinal crypts. Ultimately, this process can lead to obstruction of both the small and large intestines. In the hepatobiliary system, defective hepatic ductal salt (Cl^-) and water secretion causes retention of biliary secretions and focal biliary cirrhosis and bile duct proliferation in ~25-30% of CF patients. The inability of the CF gall bladder epithelium to secrete salt and water can lead to both chronic cholecystitis and cholelithiasis.

II.A.2.c. Sweat Gland:

CF patients secrete nearly normal volumes of sweat into the sweat acinus but are unable to absorb NaCl from sweat as it moves through the sweat duct (2,22). The defect in ductal function represents the inability to absorb Cl^- across the Cl^- -impermeable CF ductal epithelia.

II.A.3. Clinical Aspects

Most CF patients present with signs and symptoms of the disease in childhood (1). Approximately 10% of patients present within the first 24 hr of life with gastrointestinal obstruction, termed meconium ileus. Other usual presentations within the first year or two of life include respiratory tract symptoms, most prominently persistent cough and/or recurrent pulmonary infiltrates, and failure to thrive. The symptoms increase with age, and major complications are more common in older individuals.

II.A.3.a. Respiratory Tract:

Upper respiratory tract disease is almost universal in CF patients. Chronic sinusitis is common in childhood and leads to nasal obstruction and rhinorrhea. The occurrence of nasal polyps approaches 15-20% and often requires surgery.

In the lower respiratory tract, the first symptom of CF is cough. With time the cough becomes continuous and produces viscous, purulent, often greenish-colored sputum. Inevitably, periods of clinical stability are interrupted by "exacerbations", defined by increased cough, weight loss, increased sputum volume, and decrements in pulmonary function. Typically these exacerbations are treated with intravenous antibiotics (see below) with recovery of most lung function. Over the course of years, the exacerbations become more frequent and the recovery of lost lung function less complete, leading to respiratory failure.

CF patients exhibit a characteristic sputum microbiology (23). *Haemophilus influenza* and *Staphylococcus aureus* are often the first organisms recovered from samples of lung secretions in newly diagnosed CF patients. *Pseudomonas aeruginosa* is typically cultured from lower respiratory tract secretions thereafter. After repetitive antibiotic exposure, *Pseudomonas aeruginosa*, often in a mucoid form, is usually the predominant organism recovered from sputum and may be present as several strains with different antibiotic sensitivities. *Pseudomonas cepacia* has been recovered from CF sputum and is pathogenic.

The first lung function abnormalities observed in CF children, increased RV/TLC ratios, suggest that small airways disease is the first functional lung abnormality in CF. As the disease progresses, both reversible and irreversible changes in FVC and FEV₁ are noted. The reversible component reflects accumulation of intraluminal secretions and/or airway reactivity which occurs in 40%-60% of CF patients. The irreversible component reflects chronic destruction of the airway wall and bronchiolitis.

The earliest chest X-ray change in CF lungs is hyperinflation, reflecting small airway obstruction (24). Later, evidence of luminal mucus impaction, bronchial cuffing, and finally, evidence of bronchiectasis, e.g., ring shadows, are noted. For reasons that are still unknown, the right upper lobe displays the earliest and most severe changes.

II.A.3.b. Gastrointestinal:

The syndrome of meconium ileus in infants presents with abdominal distention, failure to pass stool, and emesis. The abdominal flat plate can be diagnostic with small intestinal air fluid levels, a granular appearance representing meconium, and a small colon. In children and young adults a syndrome termed meconium ileus equivalent or distal intestinal obstruction occurs. The syndrome presents with right lower quadrant pain, loss of appetite, emesis, and often a palpable mass. The syndrome can be confused with appendicitis, which occurs frequently in CF patients. The characteristic intestinal abnormalities are complicated by pancreatic insufficiency in more than 90% of CF patients. Insufficient pancreatic enzyme release yields the typical pattern of protein and fat malabsorption, with frequent bulky, foul-smelling stools. Signs and symptoms of malabsorption of fat soluble vitamins, including Vitamins A, D, E, and K, are also noted. Because pancreatic beta cells are typically spared, the appearance of hyperglycemia and a requirement for insulin is a late finding in CF and occurs in only a small percentage of patients.

II.A.3.c. Genito-Urinary:

Late-onset of puberty is common to both males and females with CF. The delayed maturational pattern is likely secondary to the effects of chronic lung disease and inadequate nutrition on reproductive endocrine function. More than 95% of male patients with CF are azoospermic, reflecting obliteration of the vas deferens (25). 20% of CF women are infertile due to effects of chronic lung disease on the menstrual cycle and thick, tenacious cervical mucus that blocks sperm

migration. More than 90% of completed pregnancies produce viable infants, and CF women are generally able to breast-feed infants normally.

II.A.4. Treatment

The major objectives of therapy for CF are to promote clearance of secretions and control infection in the lung, provide adequate nutrition, and prevent intestinal obstruction.

II.A.4.a. Lung Disease:

At present, the techniques for clearing pulmonary secretions are a combination of breathing exercises and chest percussion. A number of pharmacologic agents for increasing mucus clearance are being tested. N-acetylcysteine has not been shown to have clinically significant effects on mucus clearance and/or lung function. However, agents that degrade the high concentrations of DNA in CF sputum, e.g., human recombinant DNase, appear to be effective in decreasing sputum viscosity and increasing airflow during short-term administration (26). Experimental drugs aimed at restoring salt and water content of secretions, e.g., amiloride (27) and triphosphate nucleotides (28), appear promising.

More than 95% of CF patients die of complications resulting from lung infection. Antibiotics are the principal agents available for treating lung infection, and their use should be guided by sputum culture results. Early intervention with antibiotics is useful, and long courses of treatment are the rule. Because of increased total body clearance and volume of distribution of antibiotics in CF patients, the required doses are higher for CF patients than for non-CF patients with similar chest infections.

II.A.4.b. Gastrointestinal Disease:

Maintenance of adequate nutrition is critical for the health of the CF patient. Most (>90%) of CF patients benefit from pancreatic enzyme replacement. Capsules generally contain between 4,000-24,000 units of lipase. The dose of enzymes should be adjusted on the basis of weight gain, abdominal symptomatology, and character of stools. Replacement of fat-soluble vitamins is also required. Hyperglycemia most often becomes manifest in the adult. Principles for treating other causes of non-ketotic hyperglycemia should be employed.

For treatment of acute obstruction due to meconium ileus equivalent, megalodiatrizoate or other hypertonic radiocontrast materials delivered by enema to the terminal ileum are utilized. For control of symptoms, adjustment of pancreatic enzymes and the supplementation of intake by salt solutions containing osmotically active agents, e.g., propyleneglycol, are utilized. Hepatic and gall bladder complications are treated as for non-CF patients. End-stage liver disease can be treated by transplantation, which has a two-year survival rate exceeding 50%.

II.B. Gene therapy for CF lung disease

II.B.1. General

The concept of 'gene therapy' is potentially applicable to the treatment of cystic fibrosis lung disease. The basic premise, based on the observation that

heterozygotes (carriers) are normal with regard to lung function, is that the introduction of the complementary DNA (cDNA) coding for the normal CFTR protein will convert CF cells to the heterozygous forms that function normally. Unlike the therapeutic approaches envisioned for the treatment of bone marrow disorders, it appears to be improbable that cells can be harvested from the cystic fibrosis lung, genetically corrected, and returned to the host. The complex branching pattern of the lung would appear to preclude this approach. However, it is possible that DNA vectors can be delivered for correction directly to the lung by aerosol or direct instillation.

II.B.2. Target for CF lung gene therapy

Design of rational approaches of gene therapy for CF lung disease requires an understanding of the distribution of CFTR expression in the non-CF lung. Extensive experience with the clinical and pathological manifestations of CF suggest that the primary abnormalities in the lung relate to the defects in mucociliary clearance (see section II.A). The prediction, therefore, is that the CF gene is normally expressed in the conducting airway rather than the airspace.

The human airway is a network of branching structures that conduct air from the alveolar sacs where gas exchange occurs to the external environment (29). The general structure of the airway varies as it branches from the most proximal site below the larynx (i.e., the trachea) to the most distal terminal bronchioles. Proximal airways are lined with a surface epithelium that is pseudostratified, containing a layer of squamous cells resting on the basal lamina (called basal cells) with a variety of columnar cells including ciliated cells, goblet cells, undifferentiated cells and secretory cells with electron dense granules. Basal and goblet cells are believed to be progenitors that are capable of self-renewal and differentiation into ciliated cells.

Areas of the proximal airway surrounded by cartilage also contain submucosal glands which consist of a network of secretory tubules and associated ducts (30). These glands deliver into the airway lumen a complex seromucous secretion essential for mucociliary clearance. The most distal component of the submucosal glands consists primarily of serous tubules and serous acini. The serous cell has exocytotic vesicles that contain a mixture of bactericidal proteins. The mucous tubules, which lie proximal to the serous cells, secrete highly viscous mucous glycoproteins. All serous secretions must pass through the mucous tubules on the way to the airway. Seromucous secretions move from the tubules into collecting ducts which are lined by simple columnar epithelial cells rich in mitochondria. The high mitochondrial content of these cells suggests they may play a metabolically active role in regulating water and ion concentrations in ducts. Seromucous secretions reach the respiratory surface by passing from collecting ducts which, as extensions of the surface mucosa, are lined by a pseudostratified, ciliated epithelium.

The distal airway is normally void of submucosal glands and has a much simpler surface epithelium which is cuboidal in structure. There are essentially two types of cells in the distal airway: ciliated cells and Clara cells which contain electron dense secretory granules and are believed to be progenitors.

Critical to our understanding of the pathophysiology of CF lung disease is a precise definition of the cellular and subcellular localization of CFTR in the human airway. Previous studies have reported variable success in accomplishing this by direct localization. Immunocytochemical analyses of adult human tissues have successfully localized CFTR in epithelial cells of sweat ducts, small pancreatic ducts, kidney tubules and intestinal crypts, but failed to detect CFTR in the lung (10,11). Analysis of RNA extracted from human bronchial brushings of normal patients indicated that the CF gene is expressed at low levels in the cells released by this procedure, which were primarily ciliated and nonciliated columnar cells of the surface epithelium (31). *In situ* hybridization studies of rat trachea show low levels of diffuse staining over the surface epithelium but these studies could make no conclusions about the presence of CFTR mRNA in submucosal glands which are extremely nonabundant in the rodent airway. More recently, Zeitlin et al. detected CFTR protein in primary nasal epithelial cells by Western blot analysis (32). Function of CFTR has been clearly demonstrated in cells isolated from the surface epithelium using electrophysiological techniques [Reviewed in (14)].

Techniques of *in situ* hybridization and immunocytochemistry were used to characterize the distribution of CFTR expression in bronchus from non-CF and CF tissues (33) (see Appendix A). The predominant site of CFTR RNA and protein expression in non-CF bronchus was the submucosal glands with the highest expression detected in the ducts and serous tubules although low levels were detected in surface epithelia. Similar analyses performed on bronchi obtained from CF patients demonstrated a distribution of CFTR RNA expression similar to that found in non-CF tissue except there was markedly increased RNA expression in the collecting ducts. Expression of CFTR protein was a function of genotype where the most common mutation, $\Delta F508$, is associated with markedly decreased levels of protein in all structures, and the less common mutation, G551D allele, is associated with normal distribution of apically localized protein.

Similar techniques were used to localize CFTR in the distal airway. These studies demonstrated high levels of CFTR in a subpopulation of surface epithelial cells (approximately 10-40% of the total) in most noncartilagenous airways (data not shown).

These studies have identified a large number of possible cellular targets for CFTR gene transfer. Functional analysis and localization studies suggest that essentially all of the cells of the proximal surface epithelium and a substantial number of distal airway epithelial cells express CFTR in non-CF tissue and therefore should be targeted in gene therapy. Fortunately, these cell types are easily accessible by inhalation or lavage. CFTR-expressing cells within the submucosal glands will be more difficult to target. The current protocol, which is designed to reconstitute CFTR in surface epithelia, is based on the premise that correction of the submucosa is not necessary for some level of efficacy.

II.B.3. Strategies for Gene Therapy of CF Lung Disease

Two general approaches to correction can be envisioned: the so-called transient, or nonintegrative, expression of the CFTR cDNA; and integrative

approaches. Each general approach has its own virtues and is discussed separately. The specific rationale for adenovirus is then developed.

II.B.3.a. Nonintegrative Approaches

In transient expression approaches, the cDNA coding for CFTR is introduced into affected cells by vectors that do not require chromosomal integration for transcription. Typically, expression of the foreign protein by nonintegrative approaches will last from days to weeks. One approach envisioned is to utilize a cDNA cloned into a plasmid vector encapsulated into a liposome that can be delivered to the airway cell via inhalation. Liposomes can carry a variety of plasmid vectors, including vectors that can promote nuclear targeting of foreign DNA. Liposomal technologies for the efficient transfer of genes have improved rapidly, but at present they probably do not approach the degree of efficacy required to reach a significant fraction of airway epithelial cells (34). It is also not yet known whether there will be a requirement for selective targeting of a liposome to one epithelial cell type or another. However, it is clear that cells facing the airway lumen will require correction.

Nonintegrative therapy can also be delivered to the airway epithelial cells by a variety of viral vectors. For example, it is possible that vectors that are continually maintained episomally [e.g., Epstein-Barr virus vectors (35) can be considered. The vaccinia viral system has been utilized to express CFTR transiently in airway epithelial cells and correct the Cl^- channel defect (36); however, the fact that these viruses are ultimately lytic make them unlikely candidates for gene therapy purposes.

II.B.3.b. Integrative Approaches

The alternative strategy in gene therapy is to utilize vectors that promote integration of the foreign cDNA into the host genome. The obvious advantage of this approach is that the corrective gene can be maintained indefinitely in progenitor cells and can ultimately be viewed as effecting a 'cure' of cystic fibrosis airways disease. The most efficient vector presently available for this purpose is the retrovirus. Retroviruses deliver RNA into the host cell, which is reverse transcribed into DNA and, via retroviral integrase activities, incorporates the foreign DNA into the host genome. Recent studies have indicated that retrovirally delivered normal CFTR cDNAs correct the cystic fibrosis apical membrane Cl^- channel in polarized airway epithelial cells (37). This result, plus the observation that correction can be maintained in progenitor cells, which can differentiate into transporting epithelial cells *in vitro* for up to 6 months, indicates that this may be an efficient means of treating cystic fibrosis lung disease. The low proliferative rate of airway epithelial cells, however, makes it likely that this vector will not be sufficiently efficient to be practically useful for treating adult CF lung disease.

II.B.3.c. Adenovirus

The development of effective gene therapies for CF lung disease requires the creation of new approaches that are capable of targeting genes to airway epithelial cells by direct administration in the airway. Furthermore, gene transfer must be

accomplished in a non-replicating, stable target cell and the recombinant gene should be expressed for a prolonged period of time without pathology. In a series of studies performed in Cotton rats, Crystal and colleagues have provided convincing evidence that recombinant adenoviruses may have the properties described above necessary for safe and effective gene transfer to the human airway (38,39).

Adenoviruses are non-enveloped DNA viruses responsible for many types of self-limited respiratory illnesses in humans (40,41). Many serotypes of adenoviruses exist, some of which have been exposed to a large proportion of the population in the United States.

Adenoviruses have been the subject of extensive investigation in the laboratory (42-44). This has proven to be a powerful system for studying basic mechanisms of gene regulation. The adenovirus exists as a 35 kb double stranded DNA molecule with multiple early and late transcriptional units. The type of recombinant virus proposed in this protocol has been used as a vector system in many *in vitro* and *in vivo* settings (45). The E1 region is deleted so as to render the virus defective in replication. Under some conditions, such as high Multiplicity of Infection (MOI), E1 deleted viruses can replicate in recipient cells. The recombinant minigene is placed under the control of a heterologous promoter in place of E1 sequences.

Perricaudet and colleagues have developed E1 deleted Ad5 for use in vaccines (46). They have performed experiments in which hepatitis B surface antigen expressing virus was injected into nonhuman primates. Interestingly, no immune response was observed. Of relevance to this protocol was the study by Smith et al. in 1970 in which volunteers were exposed to type 4 adenovirus by inhalation as part of a vaccination trial (47). No substantial morbidity was noted.

II.B.4. Efficiency of gene transfer required for therapy of CF lung disease

CFTR functions as a cAMP-regulated Cl^- channel. The number of functional Cl^- channels on a per-cell basis required to account for the rates of airway transepithelial Cl^- secretion is very low (20-100 channels per cell). Thus, the number of functional CFTR molecules required per cell for full physiologic activity of the cell is small. These functional data are congruent with data reported on the level of CFTR expressed per cell on the basis of analyses of mRNA and/or protein. Studies of *in situ* hybridization reported above from our own laboratories observed that the level of expression of CFTR transcripts in the superficial epithelial cells was very low [(33); see Appendix A]. These data are complemented by the studies of Crystal and co-workers, who, using PCR-based techniques, have also found that the number of CFTR transcripts per cell is extremely low [as low as one to two transcripts per cell (31)]. Finally, these data are complemented by studies of distribution of CFTR protein in airway epithelial cell lines. In studies in which CFTR was recognized by Western blot analysis, large numbers of cells (more than 10^6) were required to produce sufficient plasma membrane mass to recognize CFTR by immunologic techniques (48). Taken together, these data suggest that relatively little CFTR per cell must be introduced to effect restoration of normal Cl^- transport in CF cells.

A second issue relates to the fraction of cells within an airway region that must be corrected to restore normal electrolyte transport to airway epithelia. Recently, our laboratory reported findings that provide an estimate of this efficiency (49). In brief, using immortalized CF tracheal epithelial cells (CFT1 cells) that maintain the CF airway epithelial phenotype with a high degree of validity, aliquots of cells were either corrected with the introduction of the CFTR cDNA by retroviral techniques, or "marked" by the introduction of the truncated IL2R receptor, which permitted quantitation of cells that exhibited the CF phenotype by cell surface labelling (anti-IL2R antibodies) techniques. These cells were admixed together in defined percentages, allowed to re-form functioning monolayers on collagen matrices *in vitro*, and assayed for restoration of Cl^- transport. As summarized in the manuscript in Appendix B, it appeared from these studies that a degree of correction could be effected when as little as 3% of the cells in the monolayer were corrected. Correction of 10% of the cells routinely produced full correction of electrolyte transport in CF epithelia. The mechanism by which the amplification of correction to adjacent cells was effected was investigated. In a series of studies designed to test the hypothesis that cell-cell communication via gap junctions may account for this phenomenon, the degree of gap junctional coupling between cells in the monolayer was assessed by direct injection of lucifer yellow. These studies revealed that each cell was coupled to approximately 10 to 15 adjacent cells. Thus, it appears that the mechanism for the so-called "bystander" effect with respect to correction is cell-cell coupling. These data likely can be extrapolated to the *in vivo* situation because a similar degree of cell-cell coupling has been reported for intact airway epithelial cells by freeze-fracture techniques.

II.C. The nose as a model for CF epithelial dysfunction

Cystic fibrosis is an epithelial disease. Ample evidence has accrued that many of the functions and activities of the upper (nasal) and lower respiratory epithelium are very similar. Morphologically, the upper and lower respiratory mucosal surfaces have a similar distribution of epithelial cell types in the superficial epithelium, i.e., ciliated, goblet, and basal cells, and similar complements of submucosal glands (50). A fundamental activity of the epithelia in both these regions, i.e., mucociliary transport, requires coordination of ciliary beat frequency, secretion of a biorheologically favorable mucin, and regulation of a periciliary liquid layer to an appropriate depth and low viscosity (51,52). This latter activity appears to be regulated by ion transport systems located in the superficial epithelia, which, upon direct comparison, appear to be similar in nasal and lower airway regions (53-55).

The ion transport activities of respiratory epithelia are "electrogenic", i.e., they are associated with the development of transepithelial electrical potential differences. A major advance for the clinical investigation of CF was the development of techniques to measure this bioelectric correlate of ion transport, the transepithelial electric potential difference (PD), *in vivo* (53,54). The capacity to make this measurement *in vivo* allowed investigators to bridge data developed from *in vitro* Ussing chamber techniques to those directly manifested in the airway epithelium *in situ* (17,56). The development of the *in vivo* PD technology permitted the first definitive identification of an epithelial defect in the lung in CF by members

of this proposal (57). In brief, it was found that the transepithelial electric potential difference *in vivo* of patients with CF was routinely 2 1/2 fold higher than that of normal or disease control patients. A large series of studies of CF lung resulted from the development of this technology. First, it was found that on a population basis the raised nasal PD discriminated very accurately between patients with CF and those with other lung diseases (17). At present, it appears that this technique discriminates better than the sweat Cl^- test in a wide variety of patients. Second, through the use of specific ion transport inhibitors and ion substitution protocols, the major defects in electrolyte transport in the superficial airway epithelium consequent to mutations in the CFTR gene were uncovered (58). In brief, the protocols that applied amiloride and substitution of Na^+ ions on the luminal surface of the nasal cavity revealed the evidence for accelerated Na^+ transport. Similarly, the bioelectric responses to luminal Cl^- substitution and the failure to respond with the bioelectric correlate of Cl^- secretion to superfused β -agonists in the nasal cavity revealed the first evidence for the airway epithelial Cl^- secretory defect in CF. Finally, the technique is presently being used to explore genotype/phenotype interactions, focussing on the importance of defects in ion transport as the mechanistic link between these two levels of disease description.

Studies of the nasal epithelium have provided a major impetus for the development of novel drugs targeted at rectifying the ion transport defects of CF. The first of the drugs targeted at abnormal electrolyte transport, the Na^+ channel blocker amiloride, was first identified as a potentially important therapeutic agent in a series of *in vivo* studies of nasal epithelium in normal subjects and CF patients (17). The initial observations were made with amiloride superfused on the superficial nasal epithelium *in vivo*. These data were confirmed and extended in studies that defined amiloride's actions as an inhibitor of excessive Na^+ absorption in freshly excised CF nasal mucosa *in vitro* (15). Based on these leads from the nasal cavity, subsequent studies in animals showed that amiloride conserved water on airway surfaces and increased the hydration of secretions, and that these actions were without safety concerns (59-61). Finally, these studies led to a clinical trial of amiloride for the treatment of CF lung disease, which, in preliminary studies, appears to be promising (27).

A similar paradigm has been followed for the investigation of novel therapeutic agents targeted on the plasma membrane Cl^- secretory defect. In brief, studies with nasal epithelium, both *in vivo* and *in vitro*, identified the profound actions of extracellular triphosphate nucleotides on inducing Cl^- secretion in CF patients as well as normal subjects (28,62,63). Again, these studies have led to a subsequent long series of studies focussing on the efficacy of these compounds in the lung and safety issues. Preliminary studies investigating these issues in CF patients are currently underway.

The nasal epithelium has also been used for studies of the response of mucosal surfaces to potentially toxic substances. The response of the nasal surface to potentially pathogenic viruses, e.g., rhinoviruses, has been investigated in a series of studies designed to determine the site of rhinoviral replication and the inflammatory and immunological responses mounted to this virus (64). These types of studies typically involve inhalation of virus followed by repetitive lavages of nasal

mucosal surfaces, often complemented by superficial mucosal biopsies. Further, the effects of other types of toxicants, e.g., O₃, have been investigated in nasal surfaces (65). Again in these studies, the toxicant is typically delivered by nasal inhalation, followed by a series of lavage and biopsy studies focussed on mechanisms of toxicity and the direct and immunologic responses to these types of injury. Finally, the nasal mucosa have been long used as a model for allergic responses to inhaled foreign antigens and other noxious stimuli (66,67). In these studies, a series of potential allergens, e.g., ragweed allergens, are instilled into the nasal mucosa, and the allergic inflammatory response followed by serial lavage measuring the release of mediators, inflammatory cells, and desquamated epithelial cells. These studies again have been supplemented by biopsy studies designed to measure the inflammatory response in individual cell types, utilizing a combination of immunocytochemistry and PCR-based technologies.

In summary, the nasal mucosa is an excellent model for the respiratory epithelium of the lower airways. Large numbers of studies have documented that the normal ion transport physiology of the nasal and lower airway regions is very similar, and that the nasal epithelium is an accurate predictor of the inflammatory responses of the lower airways in response to inhalation/administration of agents that are toxic.

II.D. Xenograft model

A critical evaluation of the safety and efficacy of adenoviral-mediated transfer of the CFTR gene to human airway would be greatly simplified if there was an authentic animal model. Critical questions relate to the biology of E1 deleted Ad5 in the context of a human airway and parameters necessary to achieve functional correction in this structure. Most currently available animal models have limitations. A major problem is that the airway of humans differs substantially in function and anatomy from that of other species. For example, many species, including rodents, are essentially devoid of the sites of mucous production found in humans (i.e., goblet cells of the surface epithelium and submucosal glands). This may explain why the recently described murine models of CF, generated by mutating CFTR in the germ line, have only mild pathology in the lung (68). Another important consideration is the marked variation in infection and replication of adenoviruses that is observed in recipient cells from different species.

Our strategy was to develop a model based on the development of a CF human airway xenograft established in a nu/nu mouse. A schematic representation of the steps involved in the procedure are shown in Figure 2.

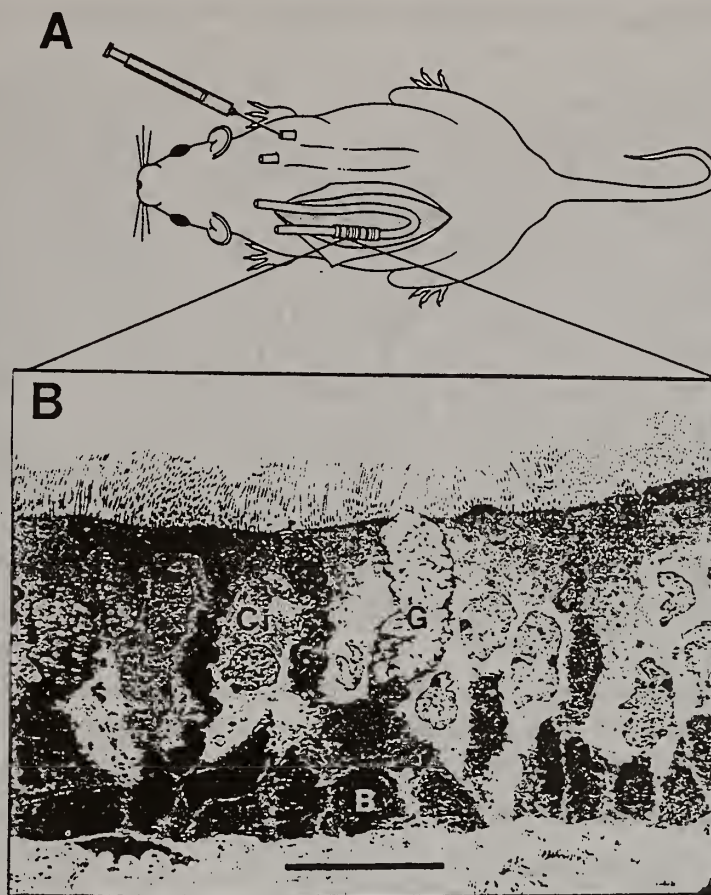


Figure 2. "Open-ended" xenografts. (A) Xenografts were ligated to flexible plastic tubing and implanted subcutaneously. Implants were sutured such that the ends of the tubing exited through the back of the neck. (B) Transmission electron micrograph of a fully differentiated bronchial xenograft epithelium. Xenograft was seeded with 2×10^6 cells and allowed to develop for 3 weeks. C, ciliated cell; G, goblet cell; and B, basal cell. (bar = 20 μ m).

Primary airway epithelial cells are released from lung tissue obtained in the setting of lung transplantation. CF cells are obtained from the bronchi of diseased lung that has been removed, and non-CF cells are obtained from proximal airway of the lung that is being transplanted into the CF patient. The cells are cultured for several days in the presence of antibiotics, released with trypsin, and seeded into the lumen of rat trachea that have been stripped of their epithelium by freeze thawing. The seeded grafts are then implanted subcutaneously into the flanks of nu/nu mice with the proximal and distal ports of the graft open to the environment via tubing ligated to the ends. The grafts are irrigated with normal saline biweekly to remove accumulations of mucus.

The grafts are allowed to mature *in vivo* for 3 weeks after which a fully differentiated pseudostratified epithelium has developed. We have now generated

108 grafts from 18 non-CF bronchial samples and 104 grafts from 13 CF bronchial samples.

The xenografts have been subjected to extensive morphometric analysis in order to evaluate the suitability of the model. Blocks of tissue from a non-CF and CF xenograft were carefully examined by transmission electron microscopy. Overall structure of the epithelium, distribution of cell types (i.e., ciliated, goblet, basal, and undifferentiated), and ultrastructural features of the various types of cells was determined. These findings were compared to similar analyses of native main stem bronchus (non-CF and CF) located adjacent to the area from which the cells destined for xenografts were removed. Electron micrographs of non-CF and CF xenografts and corresponding areas of adjacent airway showed no obvious differences in the overall structure or ultrastructural features of the various samples except that the native structure occasionally appeared taller. The distribution of cell types did not significantly differ between the non-CF bronchus and non-CF xenograft or the non-CF and CF xenograft. The CF bronchus had a significantly increased distribution of goblet cells when compared to the CF xenograft suggesting environmental stimuli may be responsible, in part, for goblet cell hyperplasia in CF (32% in bronchus vs. 24% in graft).

A detailed description of the electrophysiologic properties of the non-CF and CF xenografts are presented in section IV.A.2.d. Manuscripts describing the use of the xenograft model are contained in Appendices C, D, and E.

III. ADENOVIRUS CONSTRUCT

III.A. Vector Construction

III.A.1. Construction of pAdBgIII

The purpose of this step was to construct a plasmid containing the 5' portion of Ad 5 with deletion of E1 sequences and a unique cloning site. A series of figures (Figure 1) in Appendix F summarize this plasmid construction. As shown in Panel A, the plasmid pEHX-L3 contains sequences from Ad5 spanning map units 1 to 16.1. pEHX-L3 was digested with EcoRI and BglII and a 5.2 kb fragment isolated, which contains the adenoviral sequences from map units 9.2-16.1 and the plasmid backbone [derived from pAT 153 and described in Falck-Pedersen et al., 1989 (69)]. The adenoviral sequences from map unit 0-1 which contains the 5' inverted terminal repeat, origin of replication and encapsidation signal were amplified from the original pEHX-L3 using PCR to insert a Nhe I site immediately downstream of the EcoR I site, and a Bgl II site at the 3' end. This PCR fragment and the EcoR I/Bgl II 5.2 kb fragment were ligated to produce the plasmid pAdBgIII.

III.A.2. Construction of pAd5-CMV-lacZ

A complete minigene containing the CMV enhancer and promoter, the lacZ gene, and the SV40 late gene polyadenylation signal was cloned into the BglII site of AdBgIII. The source of the minigene cassette was plasmid pCMV- β (provided by Grant MacGregor, Baylor). A description and schematic of the construction of pCMV- β have been provided by Dr. McGregor. The description is presented below verbatim and the schematic is provided in Panel B (Appendix F).

pUC19 (70) was used as a backbone for the construction of pCMV- β . An 8mer NotI linker (New England Biolabs # 1029) was cloned into the SmaI site of pUC19. This destroys the SmaI site, but generates in the process two SacII sites, one immediately on each side of the NotI linker. This vector was designated pN. To provide a polyadenylation signal, a 196 bp fragment from the SV40 genome (nucleotides 2533-2729) was purified from an SV40 containing vector and, following the addition of BamHI linkers, this fragment was cloned into the unique BamHI site within pN. The fragment is orientated such that RNA polymerase transcribing from a promoter upstream of the NotI site passing through the NotI site and into the SV40 fragment will encounter the SV40 late gene polyadenylation signal (the early gene polyadenylation signal appears in the opposite orientation). This vector was designated pNA. A 180 bp region of the SV40 genome containing late viral protein gene 16s/19s splice donor and accept signals [obtained as a XhoI-PstI fragment from pL1 (71)] was cloned into pNA to provide the appropriate signals. This vector was designated pNAss. The human cytomegalovirus immediate early gene promoter and enhancer was obtained as a 619 bp ThaI fragment from pCM5029 (72). This was cloned into the HincII site of pUC18 and subsequently recovered as a BamHI/HindIII fragment which was treated with T4 DNA polymerase prior to blunt end ligation into the vector pNAss. The *E. coli* β -galactosidase gene from pC4AUG (73) was excised as a 3530bp EcoRI - XbaI fragment and, following the addition of NotI linkers, cloned into the unique NotI site of the vector.

The entire minigene containing the CMV promoter, lacZ gene, and SV40 polyA were excised from pCMV- β on an SphI fragment. This fragment was treated with Klenow and ligated with BclI linkers. The pAdBglII vector was digested with BglII and treated with calf intestinal phosphatase before the BclI linkered minigene was cloned in direct orientation into the BglII site of pAdBglII. See Appendix F for overview.

III.A.3. Construction of pAd5-CMV-lacZ

The purpose of this construction was to remove the CMV promoter and lacZ gene from the pAdCMV-lacZ vector and introduce a fragment containing a portion of the CMV enhancer, the chicken b-actin promoter, the full-length CFTR minigene and a small portion of retroviral sequences from Mo-MLV. A brief description of the retroviral vector from which the CFTR gene was described is provided below.

The backbone structure of this vector called pBA-CFTR includes an intact 5'LTR of Moloney murine leukemia virus (Mo-MLV) with additional Mo-MLV sequences between the 5' LTR and the internal promoter spanning nucleotide 146 at the border of U5 to the natural XhoI site in the gag coding region at nucleotide 624; see (74). The plasmid also contains wild-type Mo-MLV sequences from the ClaI site at nucleotide 7674 (which was converted to a BamHI site with synthetic linkers) to the end of the 3' LTR. Sequences containing the viral enhancer elements of the 3' LTR from the PvuII site at nucleotide 7933 to the XbaI site at nucleotide 8111 have been deleted. In addition to these sequences, there are flanking mouse genomic DNA and pBR322 sequences (spanning the HindIII site to the EcoRI site). The initial promoter used in this vector was derived from a Xho I to Mbo I fragment of the chicken b-actin gene spanning nucleotides -266 to +1 (75). The MboI site was converted to a BamHI site and the modified β -actin fragment was cloned into

the parent vector, called pgagBA. The human CFTR coding sequences were derived from a 4.6 kb *SacI* fragment which contains the complete coding sequence and small 5' and 3' untranslated regions (7). The *SacI* sites were converted to *BclI* sites and the modified fragment was cloned into the *BamHI* site of pgagBA. This vector is called pgagBA-CFTR. An enhancer was introduced into the *XhoI* site of this vector. These sequences were derived from an area 5' to the immediate early (IE) gene of human cytomegalovirus [from *SpeI* (at -580 of IE gene) to *PstI* (site in vector sequence) of CDM1 (76) were subcloned into PUC19. A portion containing IE enhancer sequences was removed on a *XhoI* (from polylinker) to *NcoI* (-220 of the IE gene) fragment [for numbering of IE enhancer see (72)]. Synthetic linkers were used to convert the *NcoI* site to a *XhoI* site and the modified fragment was cloned into the unique *XhoI* site of pgagBACFTR located 5' to the b-actin promoter. This new vector is called pCMV-BA-CFTR.

The retroviral vector pCMV-BA-CFTR was digested with *XhoI* and *NheI* to release a fragment (BA-CFTR) containing the chick b-actin promoter, the 4.6 kb human CFTR cDNA and a small portion of retrovirus-specific sequences. The plasmid pAd5-CMV-lacZ was cut with *SnaBI* and *NotI* to remove CMV promoter and lacZ structural gene, and the plasmid backbone retaining the CMV enhancer and the SV40 polyadenylation signal was blunted and ligated with the blunted BA-CFTR fragment to form the plasmid pAd5-CB-CFTR. For a schematic representation of this please see Panel C (Appendix F).

III.B. Generation of Recombinant Ad5-CB-CFTR Virus

The recombinant CFTR adenovirus was constructed from the plasmid pAd5-CB-CFTR and a modified adenovirus type 5 (Ad5), *dl7001*, in which the majority of the E3 region (map units 78.4-86) was deleted.

dl7001 was kindly provided by Dr. William Wold from St. Louis. A description of this viral genome as provide to us is presented below verbatim. *dl7001* is derived from *rec700*, an Ad5-Ad2-Ad5 recombinant which has the Ad5 *EcoRI*-A (map unit 0-76), Ad2 *EcoRI*-D (mu 76-83), and Ad5 *EcoRI*-B (mu 83-100) fragments. *dl7001* retains Ad2 sequences from map position 76 to 78.4, and it is deleted of sequences from 78.4 to about 86. In terms of the nucleotide numbering for the E3 transcription unit *dl7001* retains Ad2 sequence from nt -236 to 362 (77). The deletion extends from nt 362 in the Ad2 sequence to nt 3382 in the Ad5 sequence (nt 2437 in the Ad2 sequence corresponds to nt 2482 in the Ad5 sequence). The total deletion is 2,975 bp. Because of this deletion there are no authentic E3 AUG's or polyA sites.

pAd5-CB-CFTR was linearized by *NheI* cleavage and cotransfected with the large fragment of *ClaI*-cut *dl7001* DNA into 293 cells (a human kidney cell line containing a functional E1A gene that provides a trans-acting E1a protein) to allow homologous recombination to occur, followed by replication and encapsidation of recombinant adenoviral DNA into infectious virions and formation of plaques. Individual plaques were isolated and amplified in 293 cells, viral DNA was isolated, and recombinant adenoviral plaques containing the human CFTR cDNA were identified by restriction cleavage and Southern blot analysis. One of the positive plaques was plaque-purified a second time, and designated Ad5-CB-CFTR. This

virus was propagated in 293 cells and recovered 36-40 hr after infection by 3 cycles of freeze thawing. All viral preparations were purified by CsCl density centrifugation and either followed by gel filtration to remove CsCl for immediate use or stored at -20 °C by diluting 1:5 into a glycerol/BSA solution. Titers of viral stocks were determined by plaque assay using 293 cells.

III.C. Sequence Analysis of Recombinant Virus

Sequencing of pAd.CB-CFTR (plasmid) and adenovirus Ad.CB-CFTR (viral genome)

a. Using a primer to the moloney envelope sequence and then extending with a primer to the SV40 polyA sequence, a sequence of 434 bases has been generated. This sequence reads into the E2 region of both the plasmid and the adenovirus clone. There is 100% homology based on published sequence for the adenovirus 5 genome extracted from GenBank.

b. Using a primer to a region within the CFTR gene (starting at position 3850), a sequence of 204 bases has been generated for both clones. There is 100% homology based on the published sequence for the human CFTR mRNA extracted from GenBank. The sequence of the entire coding region of CFTR was confirmed in the precursor plasmid.

c. Using a primer to the parental vector of pAd.CB-CFTR, a sequence was generated from the EcoRI cloning site through the 5' end of the adenovirus 5 genome including the 5' inverted terminal repeat. A sequence of 282 bases was generated and based on published sequence for the 5' end of the adenovirus genome extracted from GenBank, there was 100% homology. Sequence using the same primer to the adenovirus clone is ongoing; final results are anticipated in early February, 1993 and will be forwarded when available.

IV. PRE-CLINICAL STUDIES

IV.A. Efficacy

IV.A.1. Experience with transfection of foreign genes in airway epithelia:

The first studies transducing foreign genes into human airway epithelial cells were performed in 1988 (78). In these studies, primary cultures of airway epithelial cells were electroporated in solutions with plasmids containing the neomycin resistance (Neo^R) gene. These studies showed that gene transfer could be effected by this technique but with exceedingly low frequency. Subsequently, gene targeting conjugates, containing transferrin complexed to plasmid DNA, were used to transduce airway epithelia (79). Again, gene transfer to human airway epithelial cells could be effected, but the efficiency was low. Finally, retroviruses have been used to effect gene transfer to human airway epithelial cells. Retroviral-mediated gene transfer efficiency can be quite high *in vitro* due to the large number of replicating cells. However, gene transfer *in vivo* has been of low efficiency because of the low cell proliferative rate of the normal airway epithelium (80-82). For these reasons, studies were initiated using recombinant adenovirus, which can theoretically achieve high efficiency gene transfer in non-dividing airway epithelial cells.

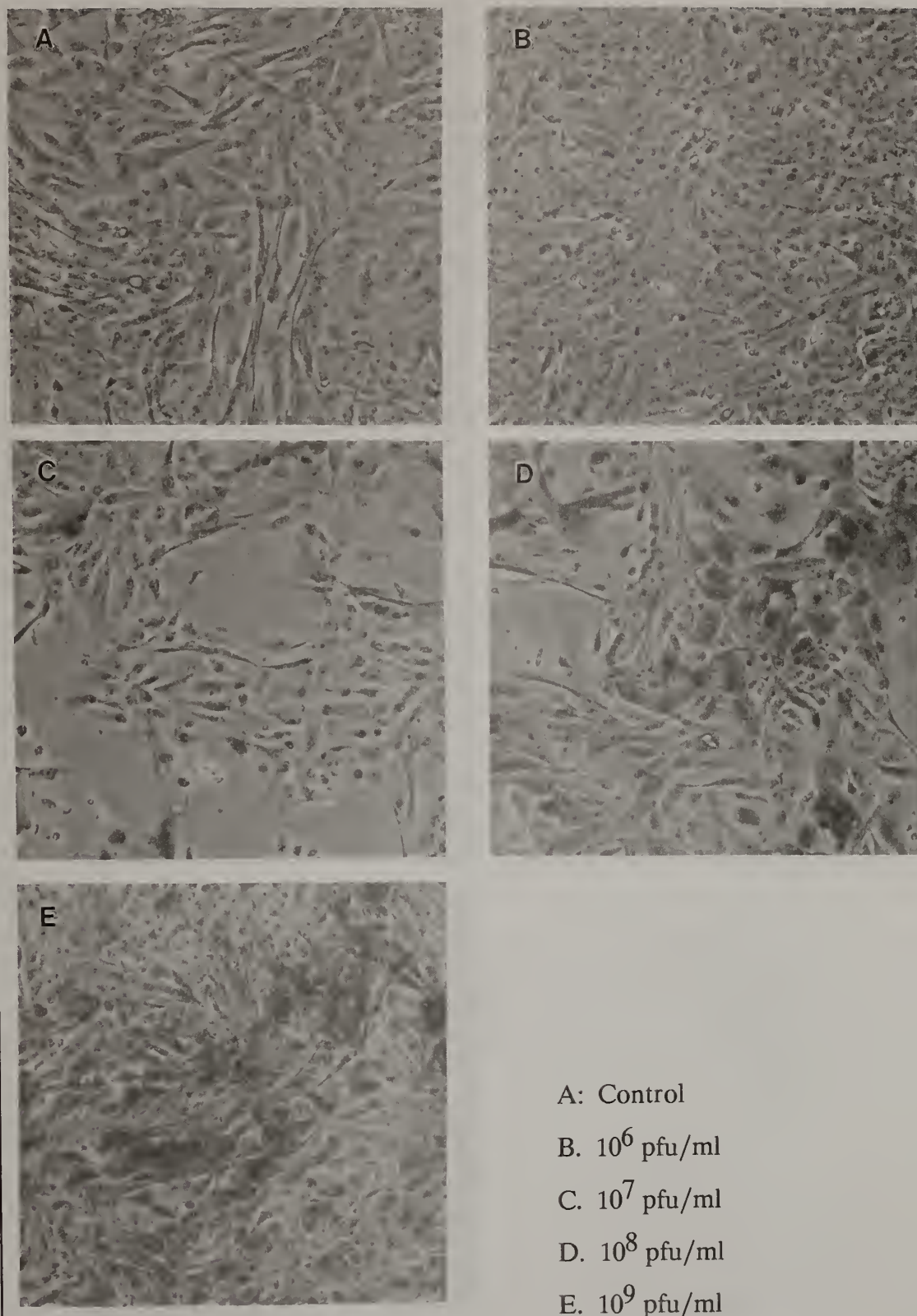
IV.A.2. Recombinant E1A-deleted adenovirus:

IV.A.2.a. Expression of reporter gene (lacZ) *in vitro*

IV.A.2.a.(i) Cultured airway epithelial cells:

For these studies, an E1-deleted adenovirus containing the lacZ marker gene under the control of the CMV promoter (Ad5-CMV-lacZ) was utilized.

The ability of this E1-deleted recombinant adenovirus to infect human airway epithelial cells was tested. Nasal turbinates were resected from normal donors and disaggregated epithelial cells cultured on tissue culture plastic in serum-free media. The cells were exposed to increasing concentrations of Ad5-CMV-lacZ (10^6 to 10^9 pfu/ml) for 30 min, and the cells stained 2 days later for lacZ expression. This relationship is shown in Figure 3.



- A: Control
 B. 10^6 pfu/ml
 C. 10^7 pfu/ml
 D. 10^8 pfu/ml
 E. 10^9 pfu/ml

Figure 3. Dose-effect relationship between pfu/ml Ad5-CMV-lacZ and lacZ expression in cultured human airway cells. (100X magnification).

These data show that $\sim 10^9$ pfu/ml is a highly effective concentration. No evidence of cell toxicity was noted at this or higher concentrations for the 30 minute exposure regimens.

Because of the availability of the CF mouse model (83,84), studies have been initiated to ascertain whether the Ad5-CMV-lacZ will infect mouse airway epithelial cells. In studies parallel to those outlined above, cultured nasal epithelial cells were exposed to recombinant adenoviruses for 30 min, and cell stained for lacZ expression. Dose-effect relationships are shown in Figure 4.

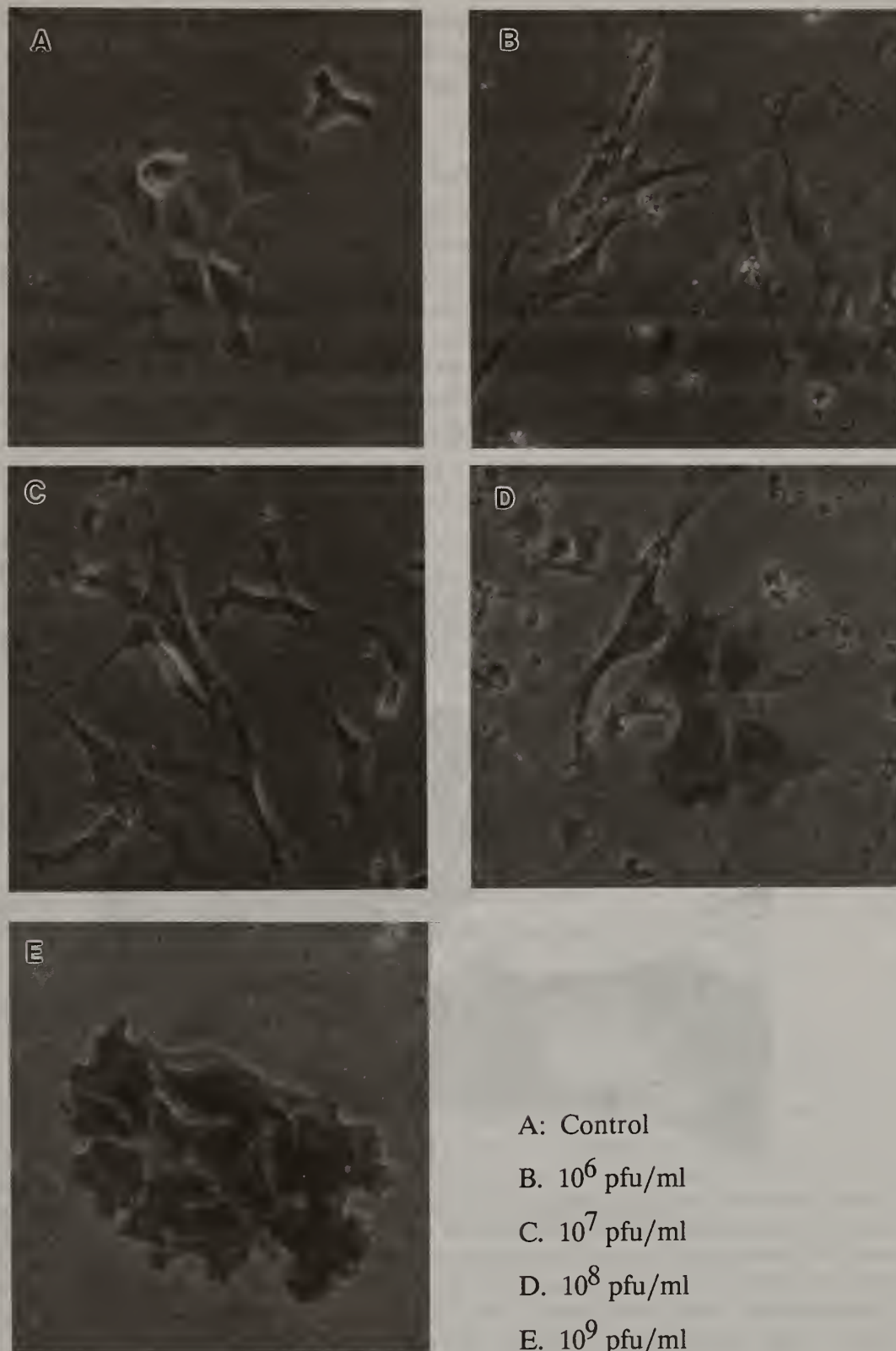


Figure 4. Dose-effect relationship between pfu/ml Ad5-CMV-lacZ and lacZ expression in cultured mouse airway cells. (200X magnification).

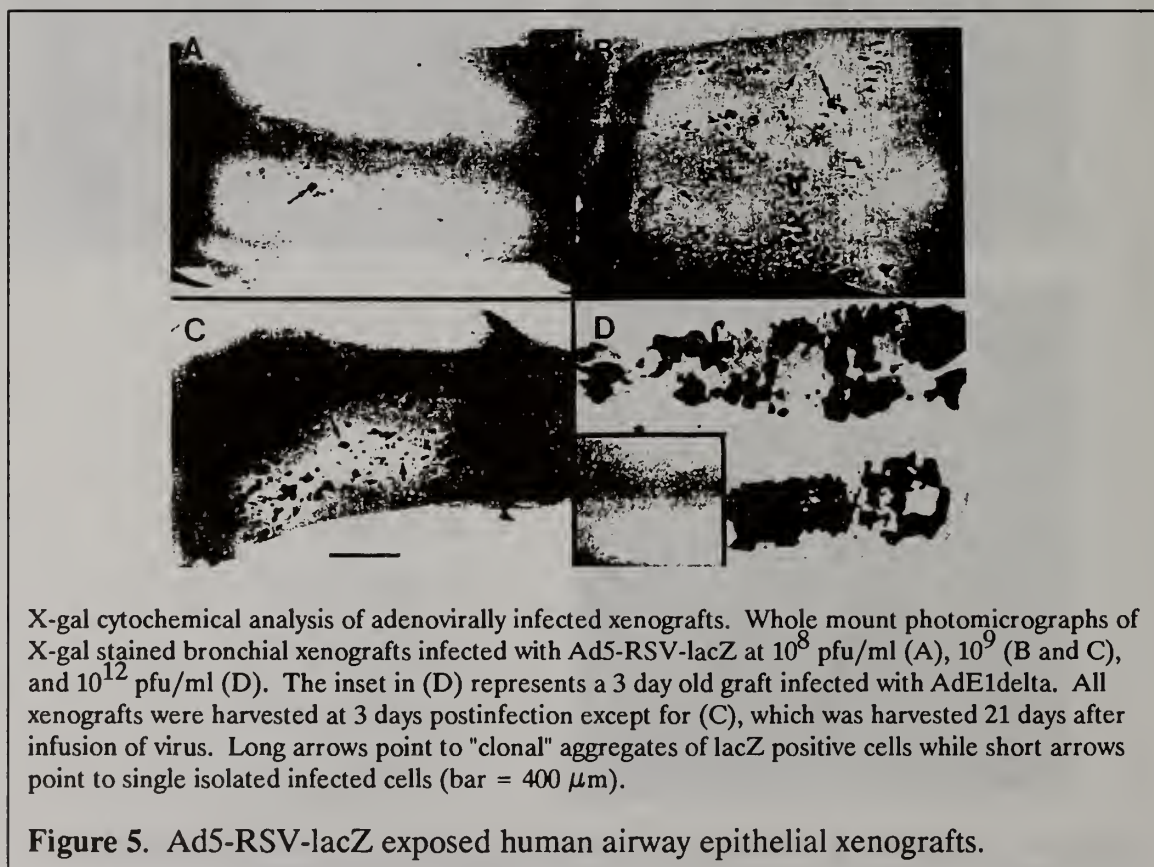
These data show that the dose-effect relationship between pfu/ml Ad5-CMV-lacZ concentrations and lacZ expression is similar for mouse nasal and human nasal epithelia. As in human tissues, effects of adenovirus on cell morphology are not observed.

IV.A.2.a.(ii) Xenograft model

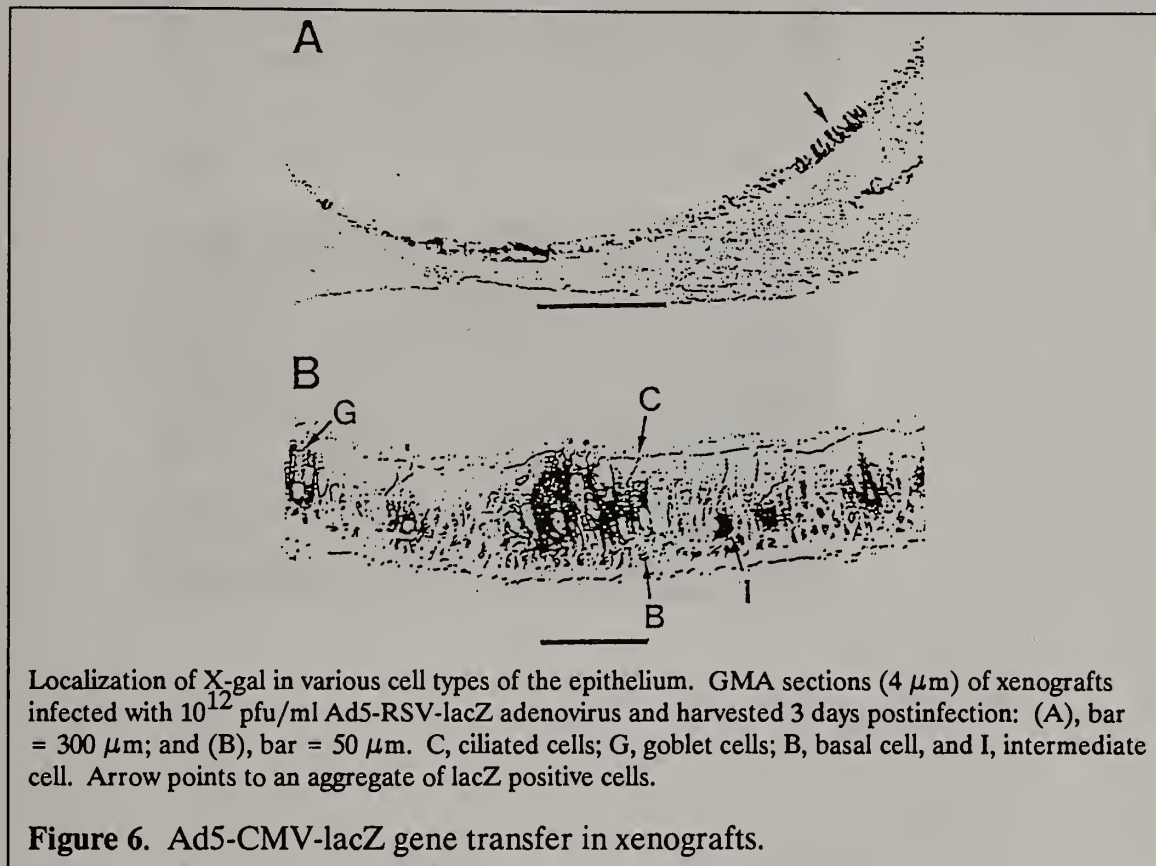
For these studies, a virus with a similar overall structure to that of the Ad5-CB-CFTR and Ad5-CMV-lacZ viruses was utilized. This recombinant adenovirus, called Ad5-RSV-lacZ, has a lacZ minigene expressed from a RSV promoter in place of E1 sequences as well as deletion of the E3 region. Into the lumen of the grafts were injected the following viruses: 1) Ad5-RSV-lacZ, 2) E1 deleted adenovirus (AdE1delta), 3) wild type Ad5, and 4) PBS ("mock"). The virus was expelled and the xenografts were subjected to the analyses summarized in Appendix E. Specific details of the experiments are provided below.

IV.A.2.a.(ii)(a) Efficiency of adenoviral-mediated gene transfer in human bronchial epithelium

Xenografts were exposed to concentrated purified stocks of Ad5-RSV-lacZ adenovirus at 10^{12} pfu/ml, 10^9 pfu/ml, and 10^8 pfu/ml for a total of 1 hour. The E1 deleted adenovirus (AdE1delta) at 10^{12} pfu/ml was used as a negative control. The grafts were harvested, fixed, stained in X-gal, and visualized enface through a dissecting microscope in order to assess the overall efficiency of infection (Figure 5).



Xenografts infected with the AdE1delta demonstrated no X-gal positive cells when harvested 3 days after exposure to virus (Figure 5D, inset), while large areas of lacZ expression were demonstrated in grafts exposed to Ad5-RSV-lacZ at 10^{12} pfu/ml and harvested at day 3 (Figure 5). Morphometric analysis of GMA sections of this xenograft indicated gene transfer in 11% of the epithelial cells (see Figure 6A and 6B below).



Xenografts infected with Ad5-RSV-lacZ at 10^9 and 10^8 pfu/ml demonstrated gene expression in 1.9% and $<0.1\%$ of the total cells of the epithelium, respectively, when harvested at three days.

Enface micrographs of these xenografts are provided in Figure 5A (10^8 pfu/ml) and Figure 5B (10^9 pfu/ml). to analyze the stability of adenoviral lacZ expression within the bronchial epithelium, xenografts were also harvested 21 days following infection with 10^9 pfu/ml Ad5-RSV-lacZ adenovirus (Figure 5C). No changes in the percentage of X-gal positive cells were seen between 3 days and 21 days postinfection (compare Figure 5B with 5C, respectively).

Similar experiments were performed with Ad5-CB-CFTR virus to assess the efficiency of recombinant CFTR expression in the xenografts. Non-CF xenografts were exposed to 10^{10} pfu/ml of virus and subsequently explanted, frozen, cryosectioned, fixed, and immunostained using the CFTR COOH terminal antibody. Figure 7 shows immunocytochemical analyses of Ad5-CMV-lacZ infected grafts (panel A - Nomarsky, and panel B - fluorescence) and Ad5-CB-CFTR infected grafts (panel C - Nomarsky, and panel D - fluorescence).

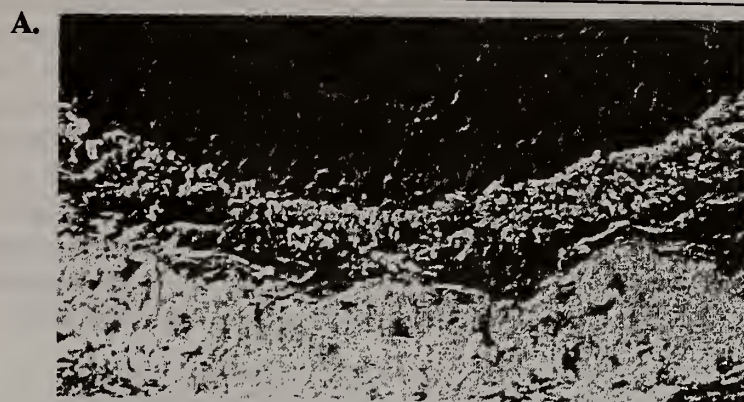
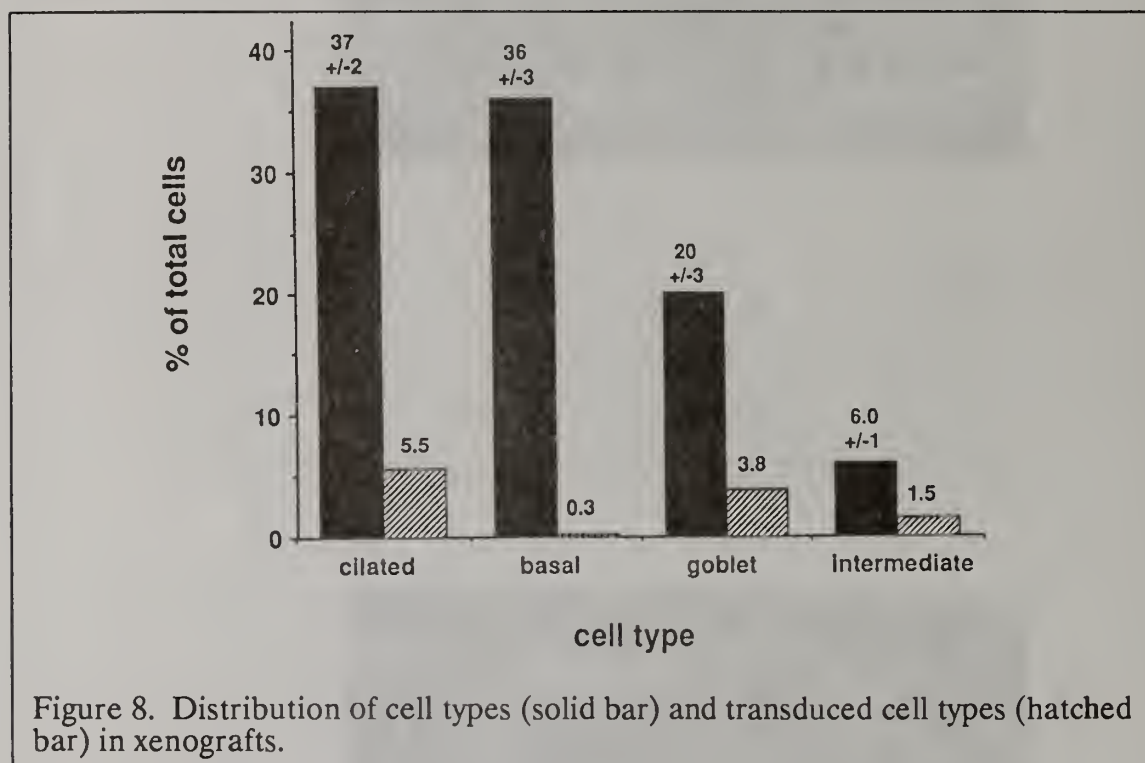


Figure 7. Xenografts exposed to Ad5-CMV-lacZ (panels A, B) or Ad5-CB-CFTR (Panels C, D).

Grafts exposed to the lacZ virus demonstrated autofluorescence below the basal lamina and low level fluorescence in basal cells representing endogenous CFTR expression (panels A and B). The grafts exposed to Ad5-CB-CFTR demonstrated a high level of CFTR expression in approximately 2-10% of the cells. Both ciliated and secretory cells expressed the recombinant CFTR, which in each case was localized on the apical basal lamina. Recombinant CFTR was also expressed in a number of intermediate cells. All types of surface epithelial cells expressed the recombinant CFTR (panels C and D). These experiments indicate that high levels of recombinant CFTR expression can be achieved in the surface epithelium. Furthermore, the recombinant protein is appropriately localized to the apical plasma membrane.

IV.A.2.a.(ii)(b) Efficiency of infection of the various bronchial epithelial cell types

Quantification of the various cell types infected by 10^{12} pfu/ml adenovirus was performed by analysis of 1,000 transgene-expressing cells detected by X-gal staining and β -galactosidase immunocytochemistry. Figure 8 shows the distribution of X-gal positive cell types as compared to the normal distribution of that cell type within the epithelium.



With a total of 11.1% lacZ positive cells in the epithelium, 5.5% were ciliated cells, 0.3% were basal cells, 3.8% were goblet cells, and 1.5% were intermediate cells. The diffusion of X-gal precipitate and the apparent non-nuclear localization of a majority of the expressing cells made the quantification of cell types within these large, highly expressing clusters difficult. We performed quantitation of lacZ positive cells by immunocytochemistry using a β -galactosidase polyclonal

antibody as confirmation of these results. In contrast to X-gal staining, which frequently produced a reaction product distributed throughout the cytoplasm, all positive cells detected by antibody staining showed a clear concentration of staining within the nucleus. The ability to detect β -galactosidase by immunofluorescence provided the opportunity to co-localize recombinant gene expression in either basal cell (cytokeratin 14) or columnar cell (cytokeratin 18) markers. Figure 9 shows a section of lacZ reconstituted xenograft incubated simultaneously with 3 antibodies: red- β -galactosidase, blue- cytokeratin 14, and green- cytokeratin 18. Of 1,500 lacZ positive cells counted, only 1 colocalized with the basal cell marker.

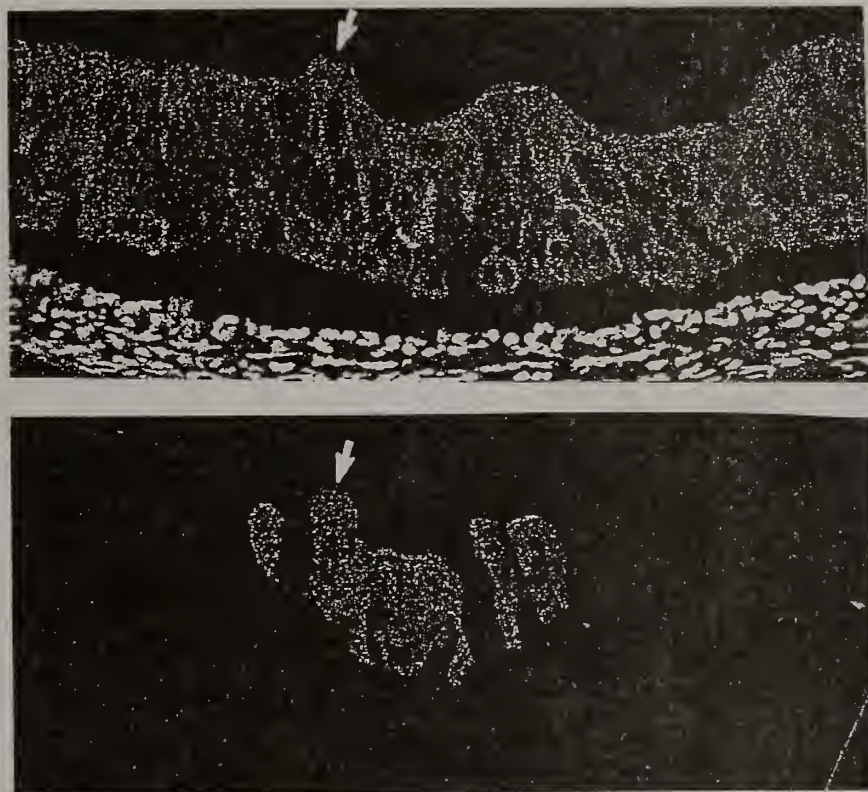
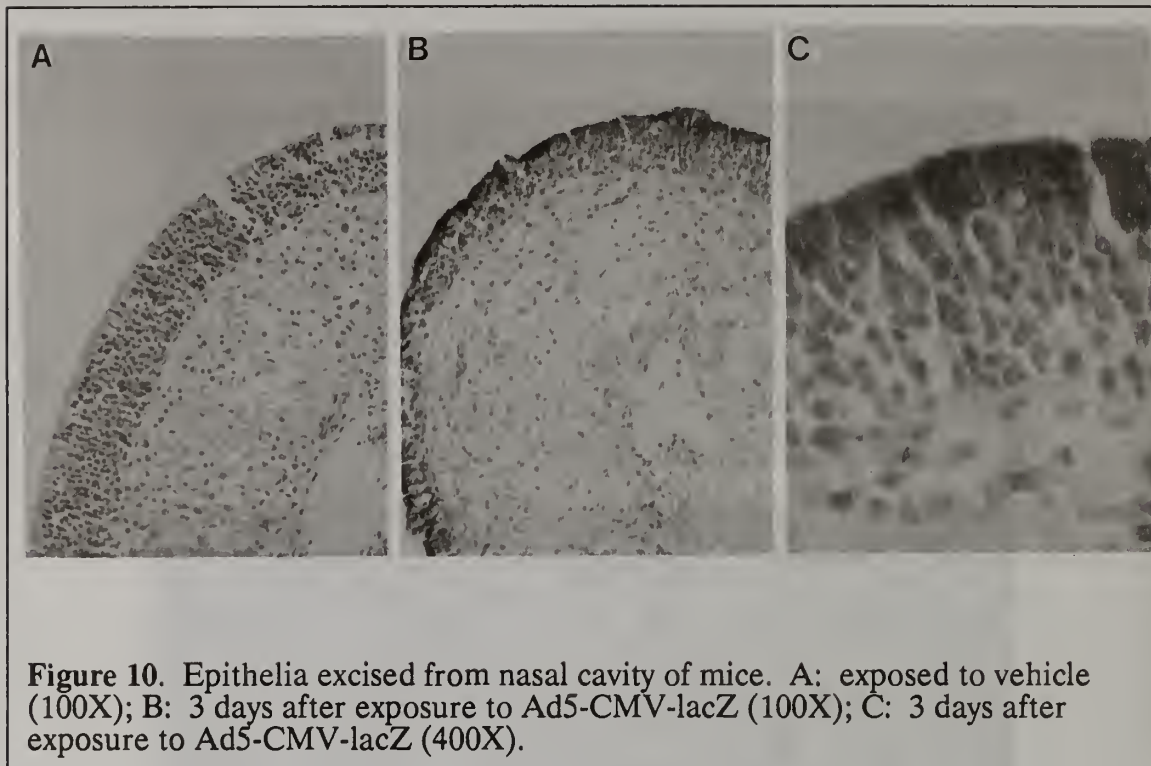


Figure 9. Ad5-CMV-lacZ gene transfer to specific cell types of xenograft.

IV.A.2.a.(iii) *In vivo* gene transfer in mice

Direct administration of E1A-deleted recombinant adenovirus (Ad5-CMV-lacZ) has been performed in the nasal cavity of mice. Patchy expression in the nasal cavity was routinely seen ($n=9$) with administration of $30\ \mu\text{l}$ of 10^{11} pfu/ml material into a single nostril (see Figure 10).



Because of the irregularity of the nasal surface area, studies were performed in the trachea where the surface area is better defined for histologic analysis. As shown in Figure 11 below, administration of $30\ \mu\text{l}$ of 10^{11} pfu/ml yields more than 50% expression of lacZ in all cell types of the trachea.

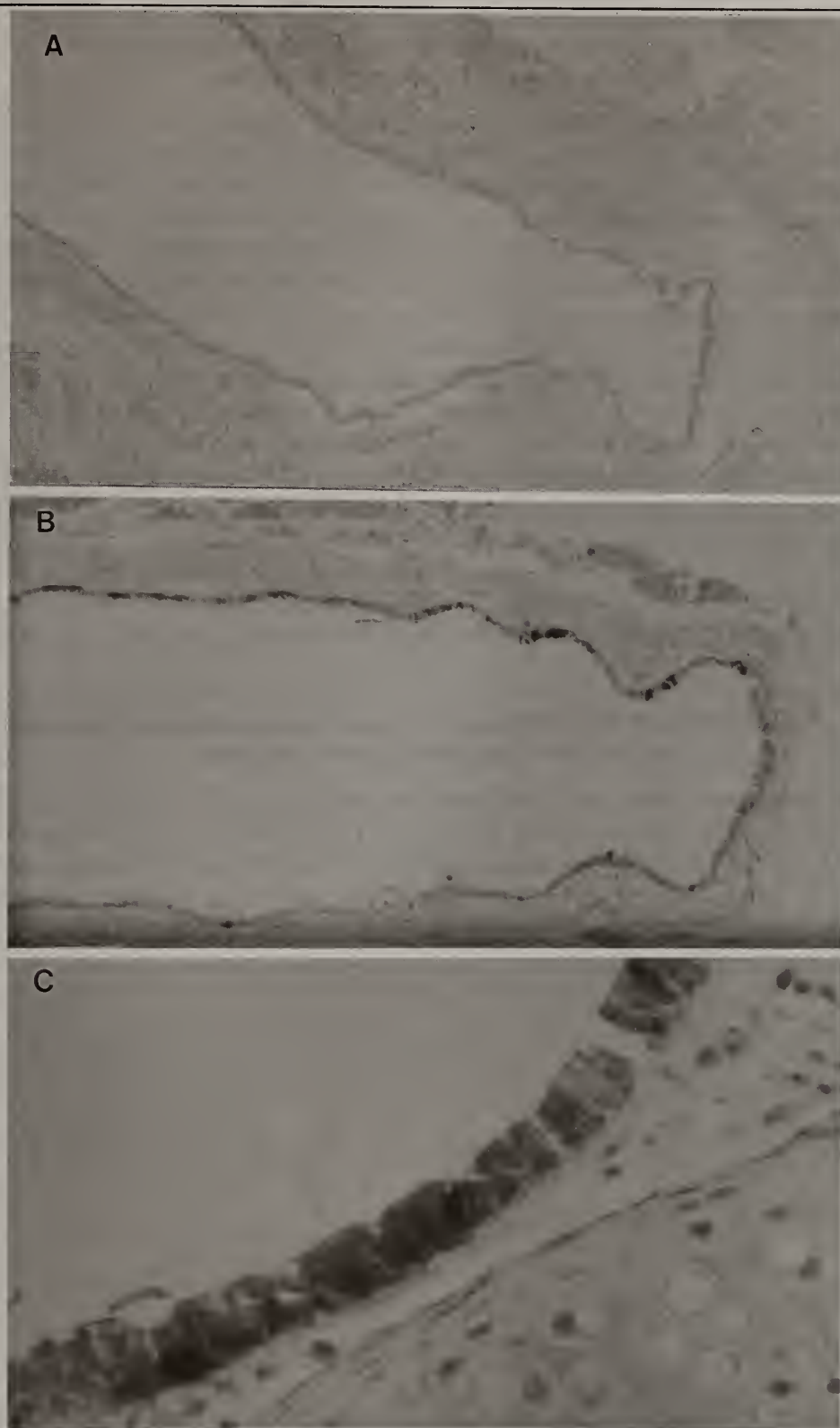


Figure 11. Tracheas from mice exposed to vehicle or Ad5-CMV-lacZ. A: control (40X); B: 3 days after exposure to Ad5-CMV-lacZ (40X); C: 3 days after exposure to Ad5-CMV-lacZ (400X).

IV.A.2.b. Expression of CFTR- phenotypic correction of CF epithelia

IV.A.2.b.(i) Correction of retrovirally transduced CFTR cDNAs of immortalized CF airway epithelia:

The CFT1 cell line is a cell line derived from a patient who was homozygous for the $\Delta F508$ mutation (85). The cells were transformed with HPV-18 E6/E7 genes. The important feature of this cell line is that it differentiates well into a polarized ion-transporting epithelium when pleuripotent cells are placed on collagen matrices that permit feeding of both sides of the epithelial cell by growth media that contains raised calcium and 3T3 cell conditioned medium. Using a murine-derived retroviral vector, functional correction of non-differentiated CFT1 cells exposed to retrovirus (LCFSN) was detected by patch clamp techniques (37). Importantly, when CFT1 cells were differentiating into polarized, ion transporting epithelial cells, correction was maintained. Thus, it appears that the CFTR protein not only can be expressed in airway epithelia but targets functionally to the apical membrane of differentiated airway epithelia. Finally, because CFT1 cells are a continuous cell line, persistence of the expression of the CFTR cDNA could be measured. It was found that after 6 months of continuous culture, CFT1 cells still express correction. However, the level of correction had diminished from the level of correction at one month. Analyses of cells indicated that all cells were still expressing the CFTR proviral cDNA, but that the level of transcription had been reduced.

IV.A.2.b.(ii) Efficiency of correction as a function of cells in a defined surface area of the airway wall:

As described above, we quantitated the fraction of CF cells that must be corrected by the transduction of the wild type CFTR cDNA to restore function to the overall epithelium (49). As shown in Figure 12 below and in the manuscript in Appendix B, partial restoration of function is observed when 3-6% of the cells in the epithelium are corrected, and full restoration of function is effected by correction of 10% of the cells in the epithelium.

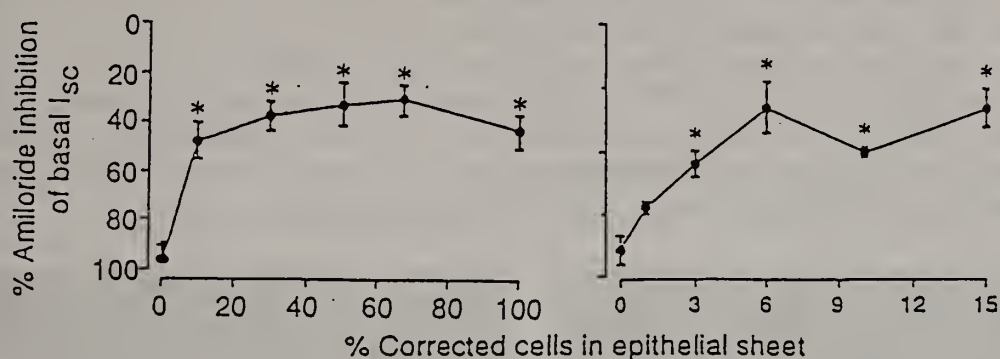
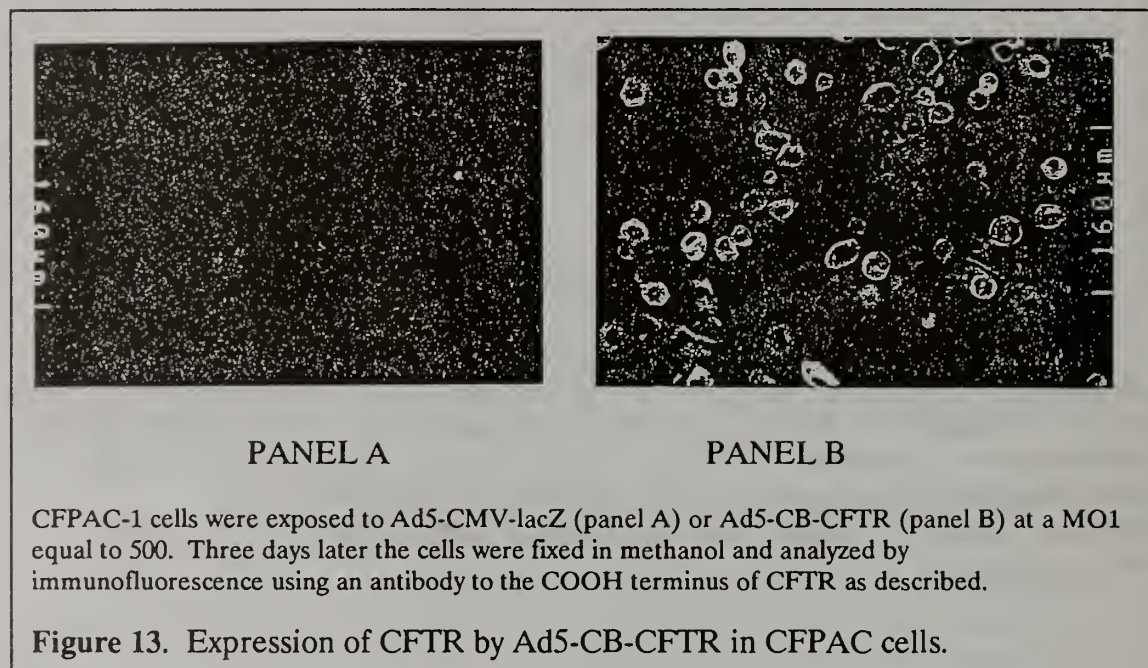


Figure 12. Relationship between percentage corrected cells and Cl^- transport.

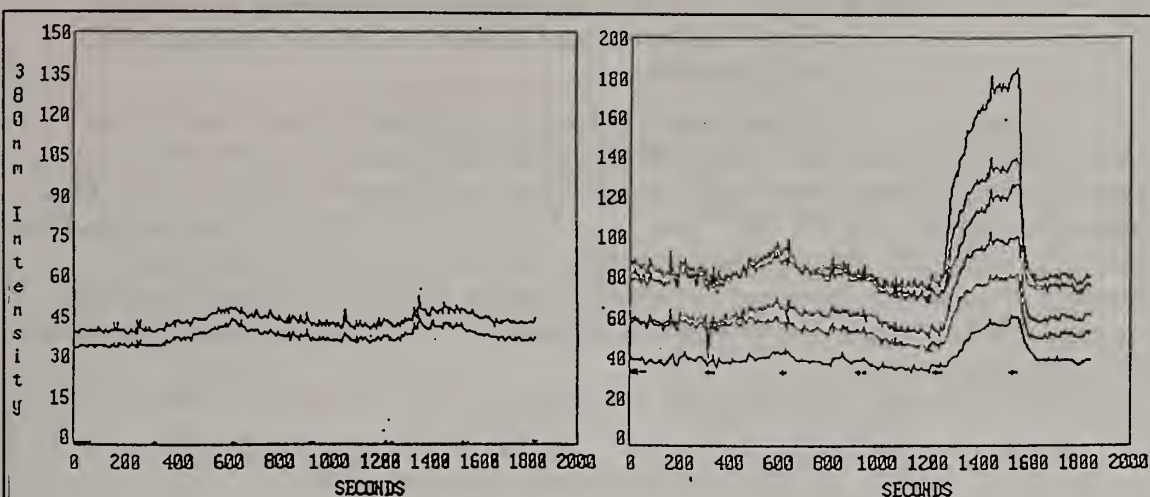
These data are derived from transformed cells in an *in vitro* situation. However, the prediction that only a low percentage of cells must be corrected likely can be extrapolated to the *in vivo* situation with adenovirus. For example, the level of expression of transduced CFTR in these studies is generally below the level transduced with the adenoviral vector. Further, the level of cell-cell communication, i.e., via gap junctions, is similar in the *in vivo* situation to that measured *in vitro*.

IV.A.2.b.(iii) Adenoviral transduced CFTR and correction - transformed epithelial cells:

The function of Ad5-CB-CFTR was evaluated in the cell line CFPAC which was derived from the pancreatic adenocarcinoma of a CF patient. Infectivity of the cell line with recombinant Ad5 was determined initially with lacZ adenovirus and subsequently with Ad5-CB-CFTR. CFPAC populations exposed to adenovirus at an MOI of 1 demonstrated expression in 30-40% of the cells. Recombinant CFTR protein expression was detected by immunocytochemistry in 100% of the cells after exposure to Ad5-CB-CFTR at an MOI=500 (see Figure 13).



The function of adenoviral-transduced CFTR protein was assessed by measuring cAMP mediated stimulation of Cl conductance using the halide sensitive fluorophore SPQ. In this assay, halide efflux is measured by an increase in fluorescence in cells loaded with SPQ and iodide. Results from mock infected CFPAC and CFPAC cells infected at an MOI of 500 with Ad5-CB-CFTR are shown in Figure 14.



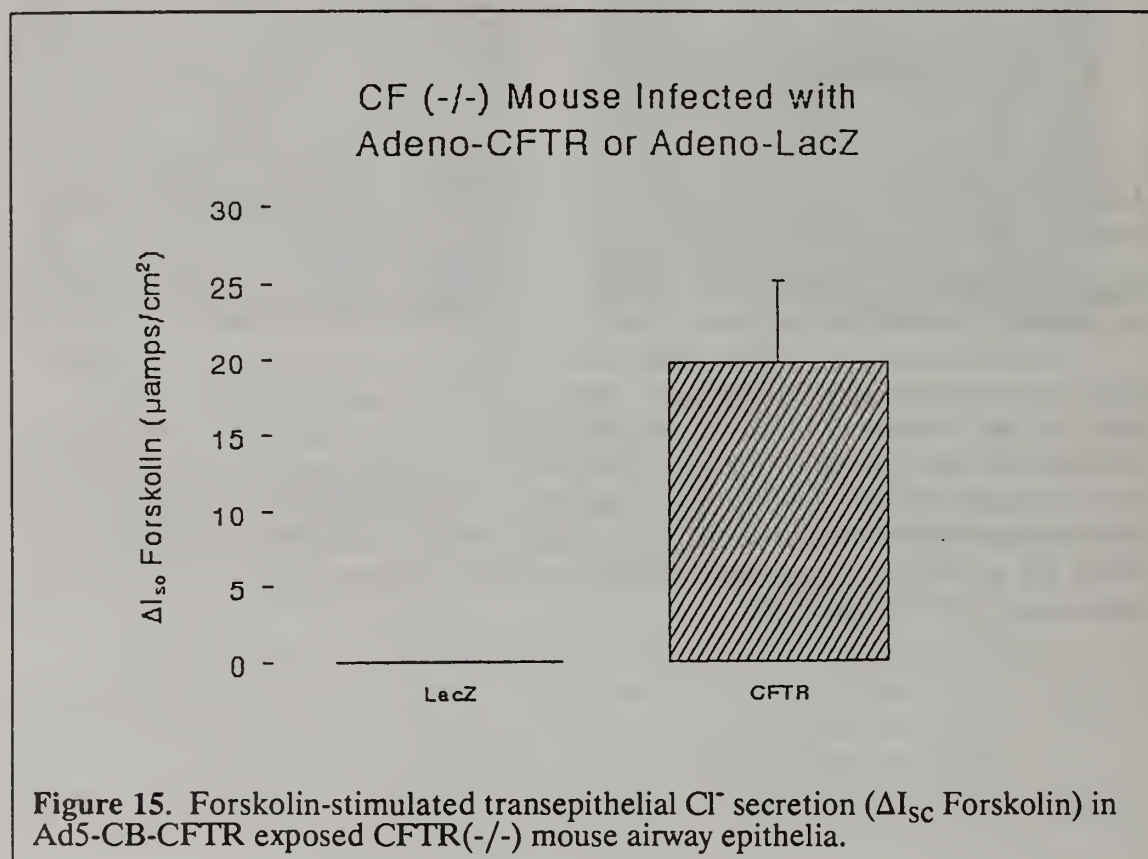
Analysis of CFPAC cells for cAMP stimulated Cl^- transport. CFPAC cells were exposed to Ad5-CB-CFTR (right panel) or Ad5-CMV-lacZ (left panel) and subsequently evaluated using the SPQ analysis. Details of this assay are provided in the text.

Figure 14. Cl^- transport in CFTR transduced CFPAC cells.

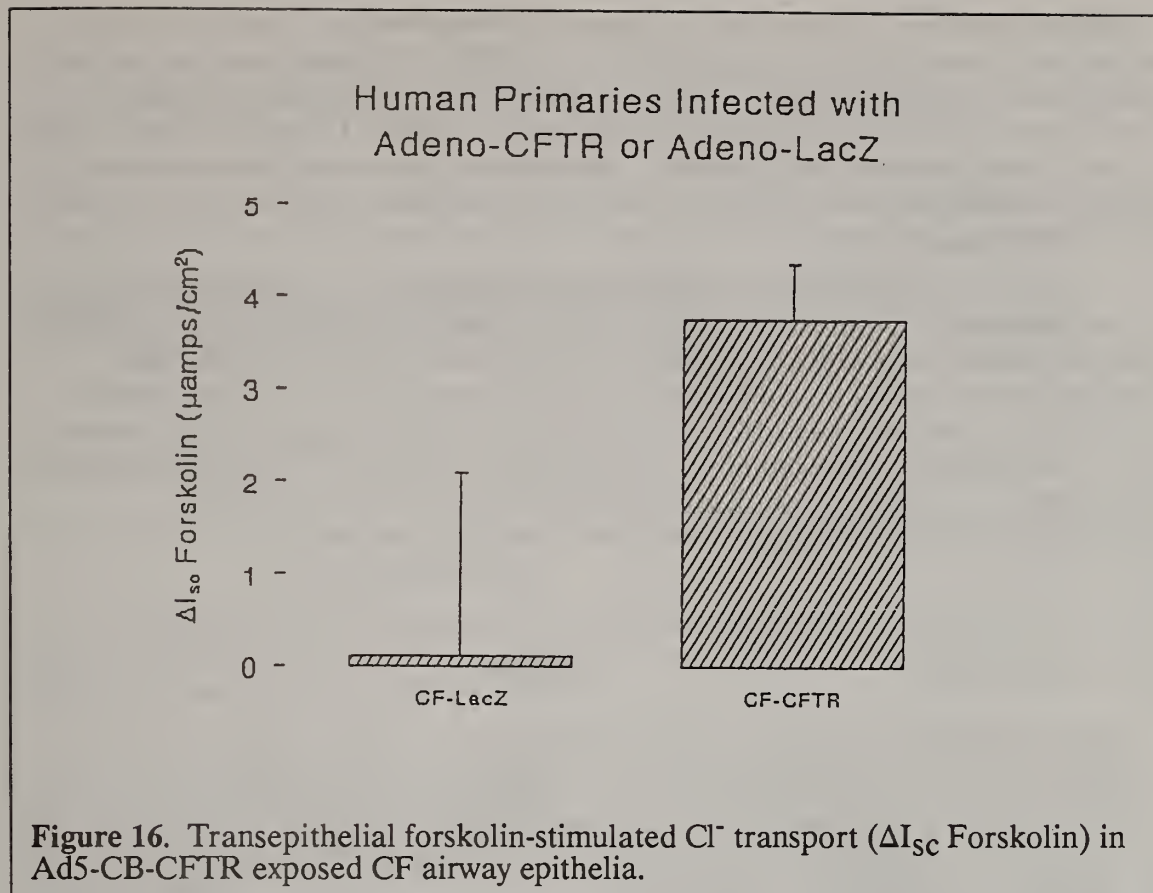
Substitution of NO_3 with iodide in the medium of each population of cells produced little change in fluorescence. Subsequent increase in the intracellular cAMP by the addition of forskolin and IBMX stimulated a rapid rise in 100% of the cells infected the wild type Ad5-CB-CFTR virus which was quenched when iodide was substituted for NO_3 . None of the cells that were mock infected or infected demonstrated cAMP mediated changes in fluorescence. Cells infected with Ad5-CB-CFTR at MOI's ranging from 50 to 500 consistently produced good SPQ responses in 100% of the cells.

IV.A.2.b.(iv) Correction of differentiated airway epithelia in primary culture from CF mice and human patients with cystic fibrosis:

Primary cultures of both nasal and tracheal epithelia from CF mice have been established on collagen matrices that allow differentiation of ion transport capacity. Preparations from both nasal and tracheal regions of CF mice have been exposed to Ad5-CB-CFTR (10^{10} pfu/ml $\times$ 24 hr; $\text{MOI} \cong 10,000$) and studied 48-72 hr after viral exposure. Phenotypic correction was assayed as the Cl^- secretory response to forskolin measured in modified Ussing chambers. As can be seen in Figure 15, full correction of forskolin-stimulated Cl^- currents is effected by the Ad5-CB-CFTR vector.



Comparable studies have been performed on airway epithelial cells excised from CF subjects and grown on collagen matrices to form functioning monolayers. In a series of studies, CF monolayers have been exposed to Ad5-CB-CFTR ($\text{MOI} \cong 10,000$) for 24 hr and bioelectric characterization performed 3 days later. Summary data are shown in Figure 16 below:



Again, as noted with mice, restoration of Cl^- transport is effected by Ad5-CB-CFTR.

Experiments were performed to test the feasibility of detecting recombinant derived CFTR in primary isolates of CF airway epithelial cells. Cells removed from the main stem bronchus of a CF patient were plated in culture and exposed to either Ad5-CMV-lacZ or Ad5-CB-CFTR at an MOI of 500. High levels of recombinant derived CFTR were clearly detected in 100% of the Ad5-CB-CFTR cells (data not shown).

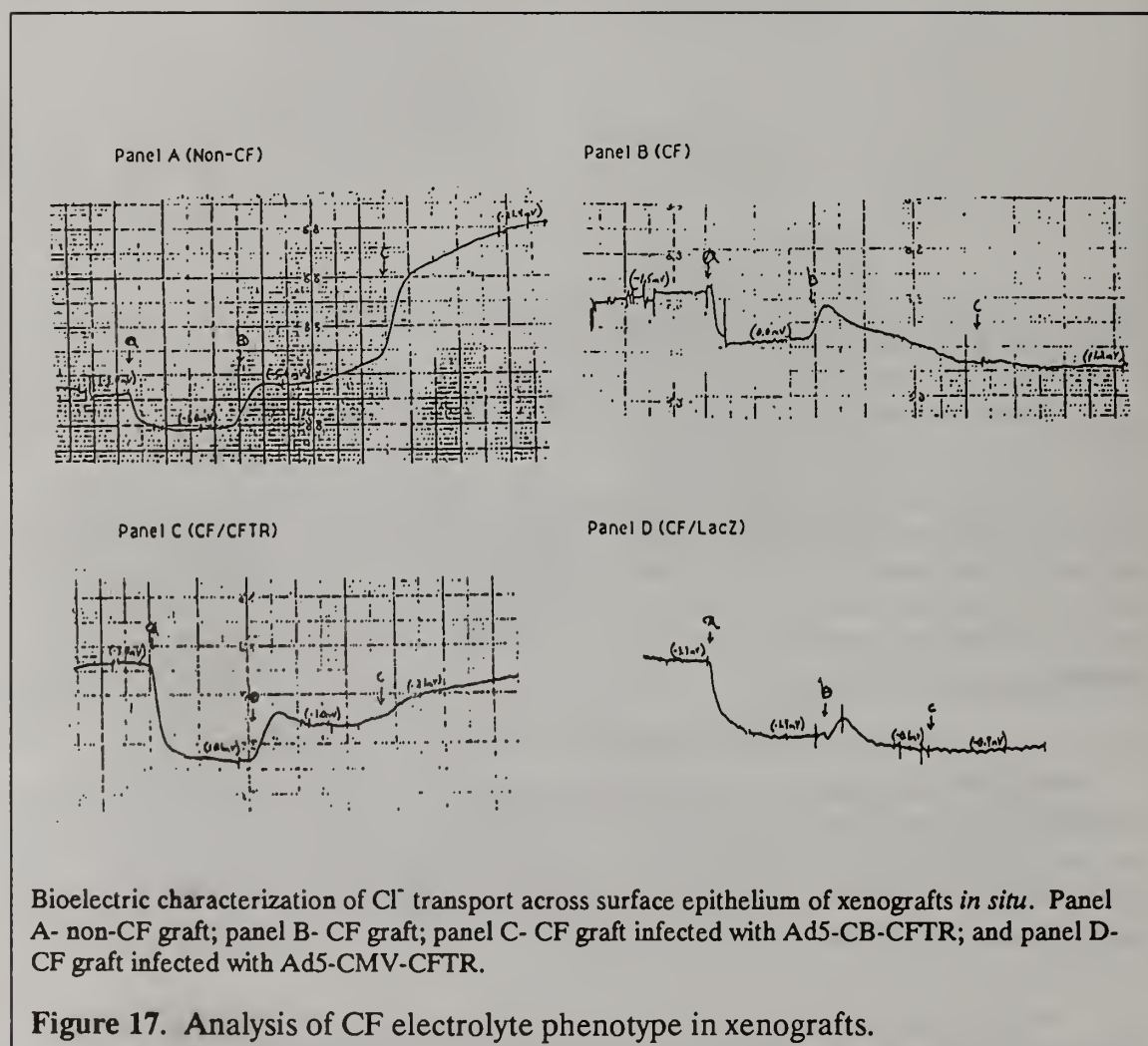
IV.A.2.c. Complementation in human CF xenografts:

In order to assess the feasibility of gene therapy it is important to determine the level of genetic reconstitution that is necessary for detectable improvement in the electrophysiological properties of the human airway. Furthermore, it is important to determine if this level of correction is possible with the virus under consideration for human trials (Ad5-CB-CFTR). This issue was initially addressed by Johnson et al. (49) and is discussed above.

We studied this question in CF xenografts exposed to Ad5-CB-CFTR virus. CF grafts were exposed to either Ad5-CMV-lacZ or Ad5-CB-CFTR and subsequently evaluated *in situ* for correction of cAMP transport across the epithelium using a modification of the procedure described by Knowles et al. (17,58). Nu/nu mice that carried xenografts were anesthetized with

ketamine/xylazine in preparation for *in situ* functional assays. The basic concept is to measure transepithelial potential difference (PD) in response to various stimuli. The ground electrode is placed subcutaneously and the measuring electrode is placed in the graft lumen bathed in the indicated solution. The initial measurement is made in the presence of 120 mM Cl^- . The luminal bath was subsequently changed in the following way: a) amiloride is added, b) Cl^- is decreased from 120 to 3 mM, and c) forskolin is added.

Results obtained with xenografts derived from a non-CF and CF individuals are summarized in Figure 17 (panels A and B respectively). Introduction of the Na channel blocker amiloride to the lumen bath leads to a drop in PD (i.e., less negative) in both non-CF and CF grafts. The PD increases (i.e., become more negative) in a stepwise manner in the non-CF graft when luminal Cl^- is decreased (representing basal conductance through CFTR) and when CFTR is stimulated with forskolin.



CF xenografts exposed to Ad5-CMV-lacZ or Ad5-CB-CFTR were subjected to similar studies. The CF graft exposed to Ad5-CMV-lacZ (Figure 17, panel D) was indistinguishable from the noninfected CF graft (Figure 17, panel B) while the

CF graft exposed to CFTR adenovirus demonstrated significant increases in PD in response to changes in luminal $[Cl^-]$ and forskolin (Figure 17, panel C).

These xenografts have been explanted and evaluated for efficiency of genetically reconstituted cells. Previous experience indicates that CFTR expression should be detected in 5-20% of the cells. Analyses of these grafts presented in Figure 17 are underway. These results indicate that single exposure of a CF epithelium to Ad5-CB-CFTR results in detectable differences in the bioelectric properties consistent with partial correction of CFTR function.

IV.B. Safety

IV.B.1. Studies of acute injury

IV.B.1.a. Studies of potential inflammatory responses in mouse nasal and tracheal mucosa in response to topically applied Ad5-CMV-lacZ were performed.

A series of studies designed to test whether Ad5-CMV-lacZ induced inflammatory responses in mouse airways have been initiated. A preliminary analysis of these studies is presented here.

Nasal cavities of mice were exposed to single ($30\ \mu\text{l}$, 10^{11} - 10^{12} pfu/ml) superfusions of Ad5-CMV-lacZ. Nasal sections were resected three days later, stained for β -gal, and evaluated for inflammatory cells (H&E). Representative samples of nasal mucosa are shown below (Figure 18).

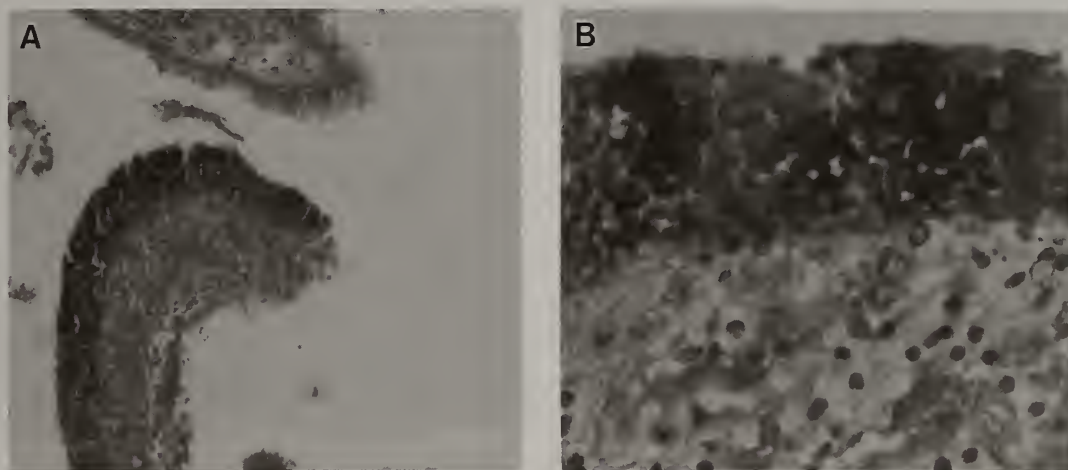


Figure 18. Nasal mucosa exposed to Ad5-CMV-lacZ and evaluated for lacZ expression and inflammatory cells. A: 100X. B: 400X.

Sections from four animals exposed to Ad5-CMV-lacZ versus vehicle were read by a veterinary pathologist (D. Meuten - see Appendix G). No inflammatory infiltrate was noted in the nasal mucosa.

Because of the more defined cylindrical surface area of the trachea, systematic studies were performed to evaluate the interaction between Ad5-CMV-lacZ superfusion and inflammatory cell infiltrates in the trachea. In an initial series of studies, the effects of a one-time exposure to Ad5-CMV-lacZ ($30\ \mu\text{l}$, 10^{11} pfu/ml) on the histologic features of mouse tracheas were studied ($n=4$). As in the nose, no inflammation was observed 3 days after Ad5-CMV-lacZ instillation. A similar series of studies was performed in which tracheas were infused three days consecutively with Ad5-CMV-lacZ and histologic evaluation performed two days after the final infusion ($n=2$). Again, no inflammation was observed. Finally, a time course study (0, 1, 2, 3, 7, and 14 days) was performed to monitor for inflammation after a single exposure to Ad5-CMV-lacZ ($30\ \mu\text{l}$, 10^{11} pfu/ml); ($n=3$ mice/time point). Representative H&E sections are shown in Figure 19. No inflammation was observed in tracheas from animals sacrificed 1, 2, 3, and 5 days after instillation. Sparse focal distribution of mild inflammation was detected at day 7, which appeared to diminish by day 14 (D. Meuten - see Appendix G).

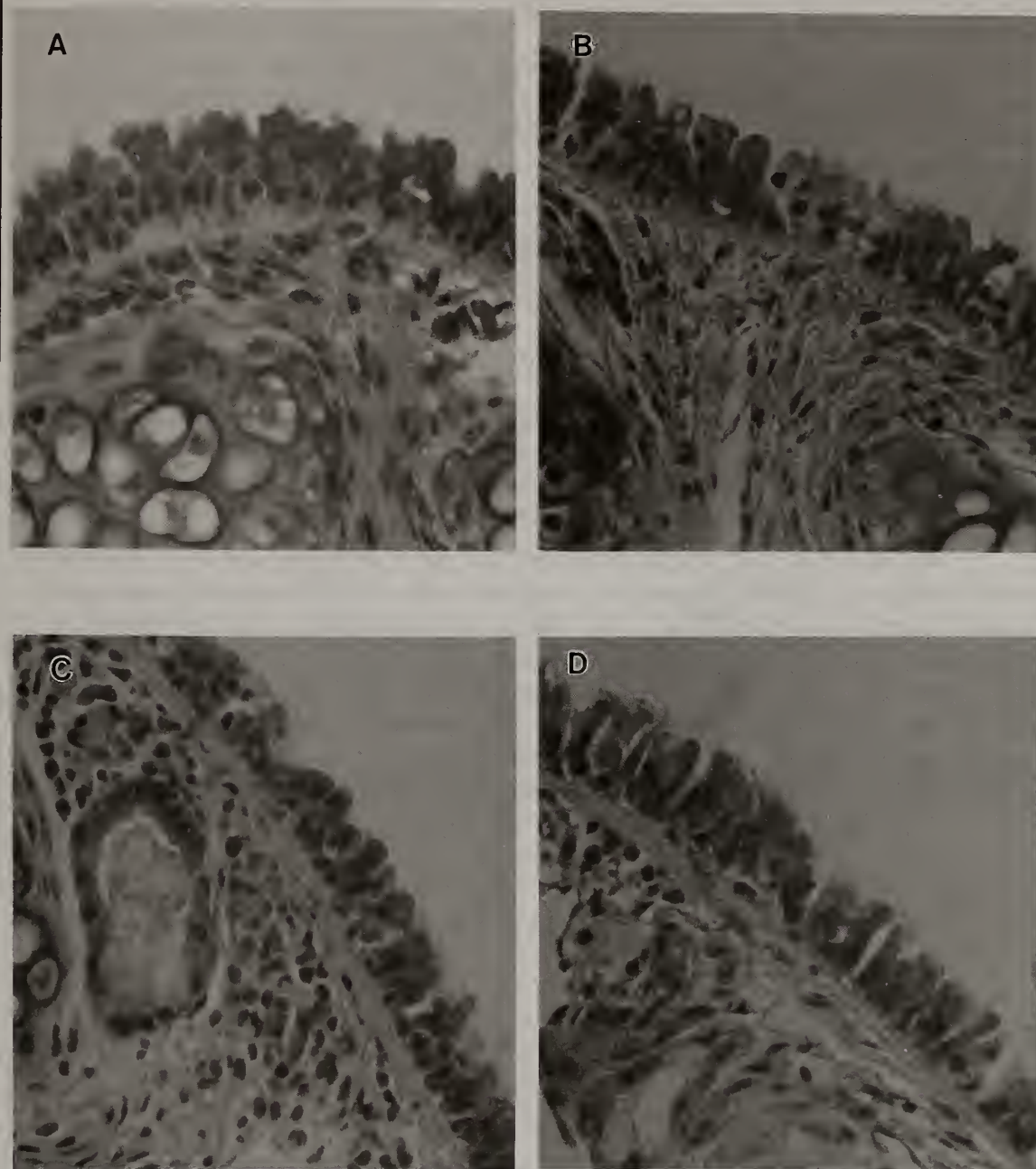


Figure 19. Tracheal mucosa sections (H&E) from mice exposed to Ad5-CMV-lacZ 3 days (Panel B), 7 days (Panel D) and 14 days (Panel C) prior to tracheal excision as compared to control (Panel A). (400X magnification).

IV.B.1.b. Studies on non-human primates

Experiments were performed in three Rhesus monkeys (*Macaca mulatta*) to further assess the feasibility and safety of gene therapy using E1 deleted

adenoviruses. Each animal was anesthetized, intubated, and ~3 ml of purified virus was administered into the airway through the endotracheal tube while the animal was lying in the left lateral decubitus position. Two animals (9037 and 9650) received Ad5-CMV-lacZ and one animal (8829) received Ad5-CB-CFTR. The animals were monitored clinically and serial blood hematology/chemistry were evaluated. Animals 9037 and 8829 were necropsied 3 days after administration of virus; animal 9650 will be necropsied 3 weeks post-infusion. A summary of the experiment is presented in the table below.

<u>Virus</u>				
<u>Animal</u>	<u>Sex</u>	<u>Wt (kg)</u>	<u>Type</u>	<u>Total (pfu)</u>
9037	Male	3.6	Ad5-CMV-lacZ	3×10^{11}
8829	Male	4.2	Ad5-CB-CFTR	3×10^{11}
9650	Male	3.2	Ad5-CMV-lacZ	3×10^{11}

The animals tolerated the procedure well without obvious clinical sequelae. Necropsy evaluations of animals 9037 and 8829 three days after administration of virus failed to reveal pathology. Blood chemistry and hematology values remained within normal limits (see Tables in Appendix H).

We have begun characterizing tissues from 9037 for evidence of adenovirus mediated gene transfer. Tissues from various organs were frozen, cryosectioned, mounted, and stained in X-gal. No staining was detected above background in brain, liver, spleen, kidney, heart, and testes. Significant staining was seen throughout the lung. Representative sections of lung are presented in Figure 20. There was patchy staining in proximal airway covering 1-2% of the surface and a majority of staining in the air space (alveolar cells and macrophages) with some reaction noted in distal airways. DNA/RNA analysis of all tissues is underway.



X-gal histochemistry of proximal airway of monkey 9037.



X-gal histochemistry of terminal bronchiole (top) and alveolus (bottom) of monkey 9037.



Figure 20. Expression of lacZ after Ad5-CMV-lacZ instillation into Rhesus monkey lung

A similar experiment (B208) has been performed in a baboon (*Papio anubis*). The specific aims were to: a) assess short term toxicity of virus administration, b) evaluate for the spread of virus beyond the lung, and c) determine the distribution and efficiency of transgene expression within the lung. In order to do this with any precision we decided to administer the virus and to necropsy the animal three days later. A lacZ virus was used to increase the sensitivity and maximize the precision of detecting transgene expression in organs. A 31 kg male baboon (*Papio anubis*) was anesthetized and 20 ml of 1×10^{10} pfu/ml of Ad5-CMV-lacZ virus was instilled

into the posterior segment of the left upper lobe via a bronchoscope. After ten minutes the virus was removed by gentle aspiration. The animal had pre-existing disease in his right lung that was evident on chest X-ray and which revealed collapse of his right upper lobe.

The clinical and laboratory features of B208 are summarized in Appendix I. The animal tolerated the procedure well without clinical evidence for acute toxicity. The blood hematology and chemistry evaluations were unchanged three days later at the time of necropsy. At necropsy, the lungs were removed en bloc, inflation fixed in glutaraldehyde, and stained with X-gal *in situ*. This provided a sensitive, specific, and comprehensive analysis of the lung for genetic reconstitution. The distribution of lacZ expression was predominantly localized to the segment that was instilled with virus. A small area of adjacent lobe contained a localized area of lacZ activity. Within the transduced lobe there was extensive expression of lacZ. The majority of cells in the alveolus, including type II and type I cells, were positive. It was difficult to assess the full extent of gene transfer into macrophages because the lungs were irrigated with PBS before fixation. LacZ activity was also found in patches in proximal airways of the segment. Low levels of activity were detected in the more distal airways. Frozen sections of other organs, including heart, liver, spleen, kidney, brain, and testes, were stained in X-gal to evaluate for possible dissemination of virus. All extrapulmonary tissues were negative. It was difficult to evaluate the lung for pathology related to the adenovirus because the animal had pre-existing disease. Diffuse atelectasis and abnormalities of the airway epithelia were noted in the lobe contralateral to the adenovirus-treated lobe which had demonstrated collapse on chest X-ray. Evaluation of tissue from the adenovirus treated segment revealed essentially normal airspace and abnormalities in the airway epithelium that were noted in nontreated tissue. A peribronchial inflammatory response was noted in some areas of the treated segment.

Similar studies of the direct instillation of Ad5-CMV-lacZ and Ad5-CB-CFTR into the nasal cavity of non-human primates are underway.

IV.B.2. Possibility of ectopic expression of Ad5-CMV-lacZ

Because of the possibility that Ad5-CB-CFTR virus could be swallowed by patients consequent to nasal administration, the ability of the adenoviral vector to transduce gastrointestinal cells was tested. In a series of studies, Ad5-CMV-lacZ was lavaged into the stomachs of mice (300 μ l, 10^{11} pfu/ml) and expression of Ad5-CMV-lacZ determined by X-gal staining in stomach, small intestine, and large intestine. In a series of 8 animals, no expression in stomach or intestines was observed.

To test for the possibility of germline transmission, a volume of Ad5-CMV-lacZ (10^{11} pfu/ml) equal to the volume to be instilled in the nasal cavity of mice was injected intravenously into male and female mice ($n=3$ each). Two days after injection, the testes and ovaries were removed and stained with X-gal. No X-gal positive cells were seen in testes or ovaries. In addition, no untoward events were seen in the animals consequent to injection of Ad5-CMV-lacZ.

In a second series of experiments, we infused lacZ adenovirus into the venous circulation of 6 male rabbits (10^8 - 10^{11} pfus) and 2 male rhesus monkeys

(10^{11} pfus). The animals were subsequently necropsied and organs were evaluated for lacZ expressing cells by X-gal histochemistry from multiple blocks of tissue. Part of this study was an extensive analysis of tissue from testes. We have not detected lacZ-expressing cells in testes from any of these animals.

IV.B.3. The effects of overexpression of CFTR

IV.B.3.a. Overexpression of CFTR in transgenic mice

Important safety concerns of this protocol relate to the consequences of ectopic and/or unregulated CFTR expression in the human lung. For example, will there be deleterious effects of CFTR expression in cells of the lung that do not normally express this gene? In collaboration with Dr. Jeff Whitsett, at the University of Cincinnati Medical Center, we established transgenic mouse lines that express human CFTR under the control of transcriptional elements derived from the human surfactant protein C(SP-C) gene (86).

The hCFTR mRNA was expressed in lungs and testes: in the lung, we found hCFTR mRNA in bronchiolar and alveolar epithelial cells and CFTR protein in respiratory and epithelial cells. While the level of expression of hCFTR mRNA varied, hCFTR mRNA and protein were detected in pulmonary epithelial cells of several lines at high levels. Lung weight, morphology, somatic growth and reproductive capacity were not altered by expression hCFTR in lung and testes of the transgenics. Our findings suggest ectopic and unregulated expression of human CFTR in lung epithelial cells may not be deleterious. A copy of the paper describing this finding is provided in Appendix J.

IV.B.3.b. Studies of overexpression of CFTR in CFT1 cells

A series of studies were designed to test whether overexpression of CFTR in CFT1 cells can lead to abnormalities in growth characteristics of CF airway epithelial cells. A clone of CFT1 cells was exposed to a MMLV-based retrovirus (LCFSN) containing the normal CFTR cDNA and the neomycin resistance gene (37). Following a 1 hour exposure, the cells were allowed to replicate for three days in culture, following which they were put under G418 selection. Cells that survived G418 selection were then pooled, and plated at clonal density on a 3T3 cell feeder layer. Individual clones were expanded, and the amount of CFTR expressed per cell quantitated by Western blot analysis. A series of clones expressing high (more than T84 cells) versus low (several fold above basal) amounts of CFTR were then identified and their growth characteristics compared to an aliquot of CFT1 cells exposed the MMLV-based retrovirus containing the truncated IL2R receptor (LISN) as a control. As shown in Figure 21, there is clone-to-clone variation in cell proliferative capacity, but no systematic relationship between this parameter and the level of CFTR expression. These experiments were repeated after activation of the expressed CFTR by forskolin, and similar results were obtained.

Growth Curves of CFT1 Cells Expressing Various Levels of CFTR

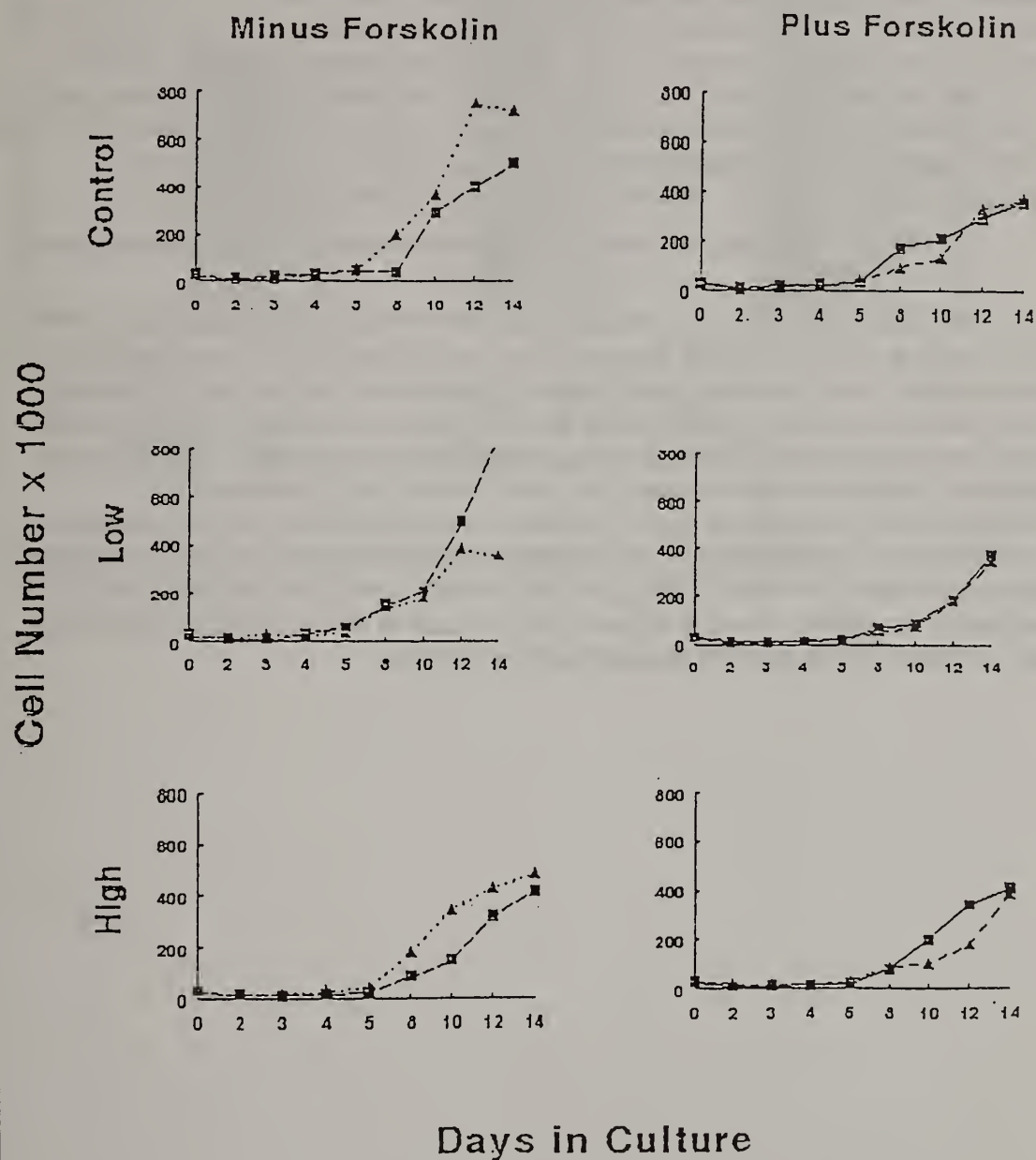


Figure 21. Effect of transduced CFTR on airway cell growth.

Finally, no systematic differences were observed in cell volume in cells expressing high quantities of CFTR, nor any inhibition of the capacity of cells to regulate their volume in response to osmotic stresses (data not shown).

IV.B.3.c. Overexpression of CFTR and Cl^- conductance in the apical membrane of airway epithelial cells

It is important to test whether Cl^- conductance in the apical membrane is regulated in part by factors other than the level of CFTR protein expression. This question was addressed in the studies of the percent correction CFT1 studies described above. As shown in Figure 12, an interesting feature is that the ability of the epithelial monolayer to secrete Cl^- is no different when 10% of the cells are corrected as compared to when 100% of the cells are corrected. These data indicate that a functioning monolayer has mechanisms to physiologically coordinate the level of apical membrane Cl^- conductance with other cellular parameters, e.g., basolateral membrane K^+ channels, necessary for regulated Cl^- secretion.

IV.B.4. Effect of recombinant adenovirus on barrier function of airway epithelia

A series of studies were designed to define whether administration of Ad5-CMV-lacZ or Ad5-CB-CFTR damaged the barrier function of airway epithelia. For these studies, nasal epithelial cells obtained from normal subjects or CF patients were plated on collagen matrices and the formation of an intact epithelial barrier measured by the development of a transepithelial resistance (R_t). The effects of adenoviral administration on day 2 in culture versus day 3 in culture on transepithelial resistance of these preparations was compared to a vehicle control. As shown in Figure 22 (below), no changes in resistance were seen in monolayers exposed to Ad5-CMV-lacZ ($\text{MOI} \cong 10,000$) or Ad5-CB-CFTR ($\text{MOI} \cong 10,000$) as compared to vehicle. Thus, it appears that in acute *in vitro* studies, recombinant adenoviruses do not perturb mucosal barrier function.

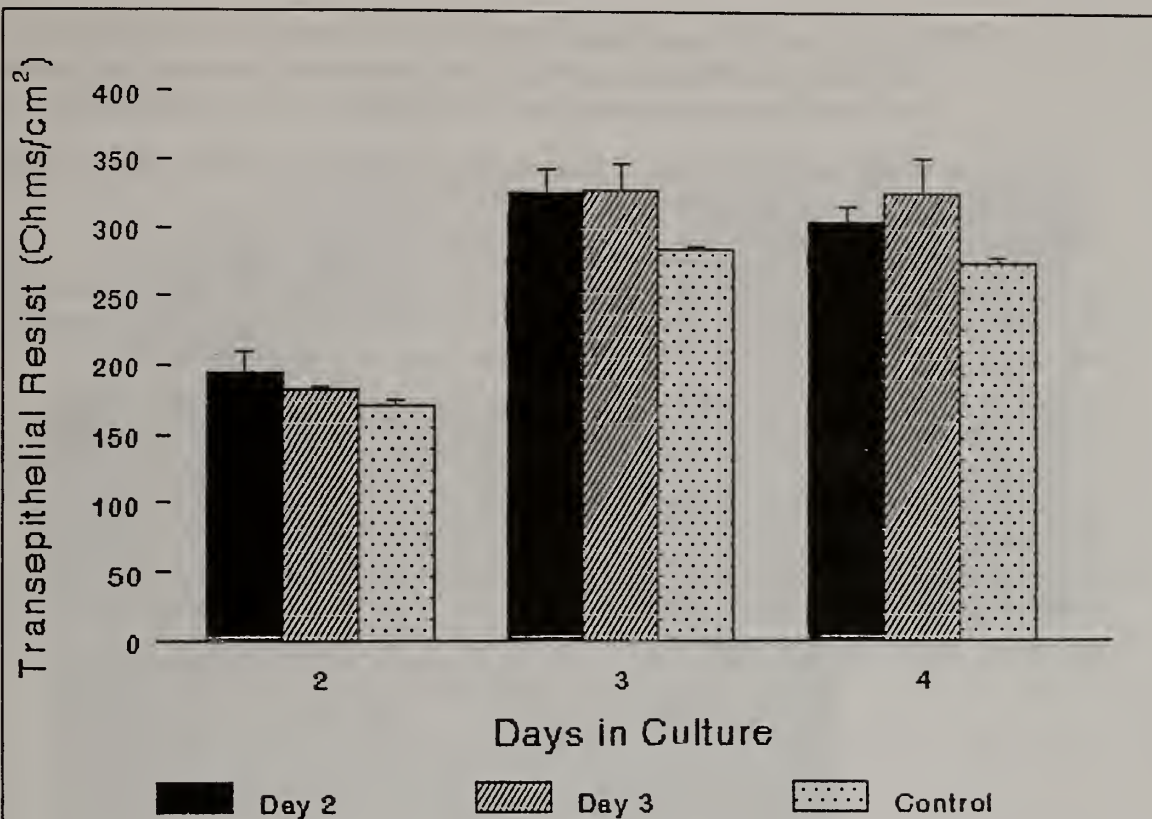
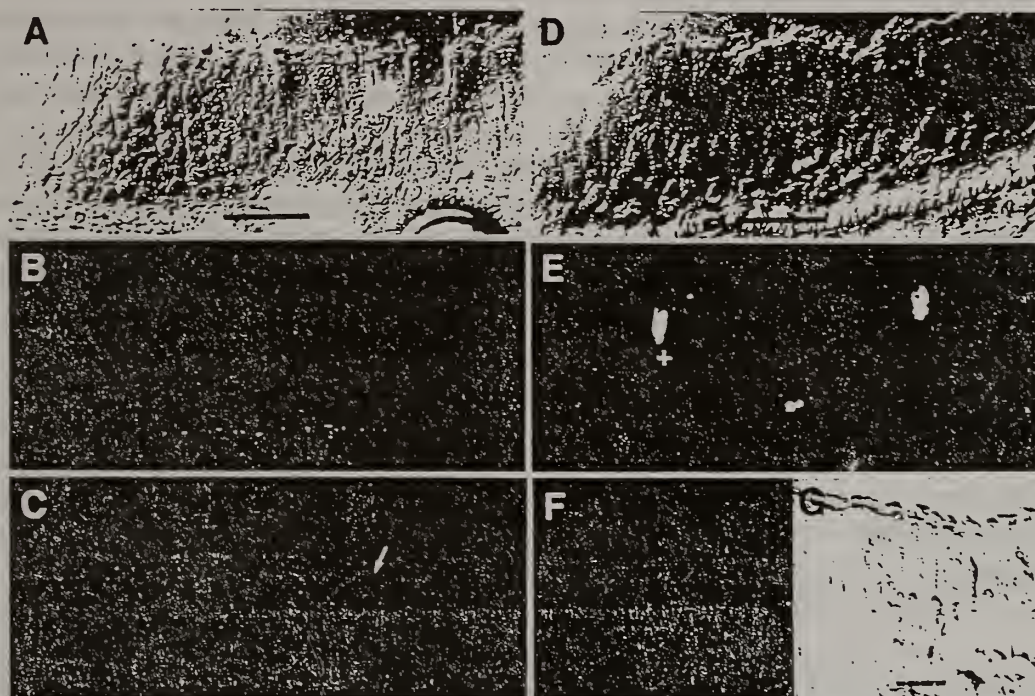


Figure 22. Effect of adenoviral vector exposure on epithelial barrier function

IV.B.5. Biology of recombinant adenovirus in human airway epithelia - including persistence, expression of adenoviral proteins, and recovery of adenovirus (see manuscript of Engelhardt et al., Appendix E)

IV.B.5.a. Expression of late adenoviral proteins (hexon, fiber) within human bronchial epithelium

Adenoviral protein expression from both wild type and recombinant adenovirus was analyzed by immunocytochemical techniques. Antibodies generated to purified adenovirus hexon protein (1056F) and purified adenovirus type 3 fiber protein (805F) have been shown to crossreact with all 43 human adenoviral serotypes. Both antibodies were directly labeled with FITC and used in double immunofluorescence studies with the polyclonal β -galactosidase antibody. Wild type adenoviral infection into xenografts produced immunoreactive cells with both adenoviral antibodies 805F (Figure 23D and E) and 1056F (data not shown) at 21 hours postinfection. In contrast, retrovirally infected grafts showed no reactivity with these antibodies (Figure 23F and G). Xenografts infected with 10^{12} pfu/ml recombinant Ad5-RSV-lacZ adenovirus showed no evidence of adenoviral protein expression within 1,500 lacZ positive cells analyzed (Figure 23 A-C).



Immunocytochemical detection of adenoviral proteins. Frozen sections ($6\ \mu\text{m}$) of xenografts: panels A-C - infected with 10^{12} pfu/ml Ad5-RSV-lacZ, harvested 3 days postinfection, and analyzed by double immunofluorescence (Nomarski, panel A) with antibodies to adenoviral proteins (805F, detected with FITC, panel B), and β -galactosidase (detected with texas red, panel C); panels D and E-infected with wild type adenovirus, harvested 20 hours postinfection, and analyzed by double immunofluorescence (Nomarski, panel G) with antibodies to adenoviral proteins (805F, detected with FITC, panel F). Nuclear localized β -galactosidase is marked by an arrow while an adenoviral protein positive cell is marked by a +. (bar = $30\ \mu\text{m}$).

Figure 23. Immunocytochemical localization of adenoviral proteins in xenografts.

IV.B.5.b. Expression of early and late adenoviral gene products in human bronchial epithelial xenografts

We have developed techniques of triple immunofluorescence to colocalize the transgene product with a variety of adenoviral gene products including: a) the E2a gene expressed in the early and late phase of the adenoviral life cycle that encodes a 72 kd DNA binding protein; and b) the L3 and L5 transcripts, formed from the single late transcriptional unit, that encodes the structural proteins hexon and fiber, respectively. The program of adenoviral protein expression in cells of the human xenograft differed substantially between wild type Ad5 and the E1 deleted recombinants. Cells infected with wild type Ad5 expressed high levels of the

structural proteins hexon and fiber and low levels of the E2A gene product, indicating that they are capable of supporting the full life cycle of Ad5. Cells harboring E1 deleted Ad5 expressed little, if any of the structural proteins hexon and fiber; however, the E2a gene was expressed at high levels in a subset of transgene containing cells. This suggests that the recombinant virus is prevented from transitioning into the late phase of transcription in the absence of E1a and E1b. However, a subset of cells are capable of activating transcription from the E2a promoter independent of E1a and E1b. The implications of this observation for the treatment of patients are unclear. The only significant concern we have is that the recipient may generate an immune response to the 72 kd product of E2a. Western blot analysis of sera from recipient animals (ferrets and primates) has failed to identify antibodies to the 72 kd protein, suggesting that this may not be a problem.

IV.B.5.c. Recovery of E1 deleted adenovirus from xenografts

We have also used the xenograft model to determine if a genetically reconstituted human airway is capable of supporting ongoing production of recombinant adenovirus. Xenografts were sequentially irrigated for a three week period after exposure to adenovirus, and the effluents were analyzed for wild type and recombinant virus. Wild type virus was never detected in the effluents and the concentration of recombinant virus dropped to undetectable levels ($<10^3$ /ml) within 14 days of initial infection.

We have since expanded these experiments to include more grafts and to increase the sensitivity of the virus assay to one particle per ml of effluent. A similar pattern emerged from these additional experiments in that wild type virus was never detected in the effluents and the amount of recombinant virus dropped at least four logs within one week of infection. However, in some of the grafts low concentrations of recombinant virus (10 - 10^3 /ml) were detected in effluents during the second phase of the experiment (7-24 days). Interestingly, there was a direct relationship between the efficiency of stable genetic reconstitution and recovery of virus in that the highest quantities of virus (10^3 particles/ml of effluent) were recovered from grafts that were most efficiently reconstituted with transgene (5-20% of cells), whereas virus that diminished to undetectable levels were from the effluents of grafts that were poorly reconstituted with transgene ($<0.01\%$ of cells). Mechanisms responsible for recovery of virus in this model are a matter of speculation. It is possible that the virus recovered in the effluents represents residual virus from the initial infusion. An alternative explanation is that the genetically reconstituted xenografts are supporting low levels of virus production. Replication of E1a deleted viruses has been described *in vitro*. One potential mechanism to account for the presence of virus in effluents is that an occasional cell in the xenograft overcomes the block in adenoviral replication, leading to its death and the production of some recombinant virus. The life cycle of group C viruses such as Ad5 in the context of full E1 expression is extremely efficient and results in the production of 10,000 virions per infected cells. Progression of the full Ad lytic cycle in only one to two cells per xenograft per week could account for the steady state level of recombinant virus detected in the effluents (i.e., 100 to 1,000 virions/irrigation). Another potential mechanism is that the virus replicates at low levels in a large population of infected cells and low quantities of virus are released

when the cells undergo normal turnover. The inability to detect wild type levels of structural proteins in a sample of 1500 infected cells is consistent with either mechanism. Because a low level of viral vector was detected in an immunocompromised model (xenograft/nude mouse), an issue is whether the vector will be recovered in a non-immunocompromised host that is permissive for viral replication, e.g., a cotton rat, and primates in the context of the proposed protocol. We are addressing this issue in experiments performed as described below.

IV.B.5.d. Recovery of E1 deleted adenovirus from cotton rat

Preliminary experiments in cotton rats indicate that gene transfer can be achieved with little inflammation. 300 μ l of 3×10^{10} pfu/ml Ad5-CB-CFTR and Ad5-CMV-lacZ were instilled by midline tracheal incision into two cotton rats (125 g males). On day 2 the animal was sacrificed and lungs were inflation fixed with 0.5% glutaraldehyde. Lungs were paraffin embedded and sectioned at 4 μ m. X-gal staining was predominantly localized in the alveolar regions with some bronchiolar staining. No evidence of inflammation was seen. Studies to assess the presence of E1-deleted adenoviral shedding in cotton rats are underway.

IV.B.5.e. Absence of recovery of E1-deleted adenovirus from baboon

The aims of this experiment (B210) were to: a) determine if recombinant virus is chronically shed; b) assess long and short term toxicity of the Ad5-CB-CFTR virus; and c) determine the efficiency and stability of CFTR gene transfer. To answer these questions we administered lacZ virus in the left lung and CFTR virus in the right lung and subsequently recovered fluid and tissue from each segment via a bronchoscope. The data are summarized in Appendix K.

The animal is a 33 kg male baboon (*Papio cynocephalus*) that was anesthetized and intubated. Ad5-CMV-lacZ (20 ml, 1×10^{10} pfu/ml) was instilled into the posterior segment of the left upper lobe and Ad5-CB-CFTR was instilled into the posterior segment of the right upper lobe. After five minutes excess fluid in the segments was removed by gentle aspiration. The animal has been evaluated three days and two weeks after treatment according to the protocol, which includes: physical exam; arterial blood gases; chest X-ray; blood chemistries and hematologies; nasal and rectal swabs and blood serum for adenovirus assay; and selective bronchoscopy of the two segments with lavage, transbronchial biopsy, and bronchial brushing.

B210 has tolerated all the procedures well without complications or signs of acute toxicity. Lavage from the lacZ segment contained Ad5-CMV-lacZ virus at three days; however, this was no longer present at two weeks. Nasal swabs, rectal swabs, urine, blood, and lavage from the CFTR segment remained free of detectable lacZ virus. The chest X-rays remain normal and arterial blood gases and blood chemistries/hematologies remain unchanged (Appendix K). The total number of cells recovered in the lavage fluid remained unchanged as did the cell differential from the CFTR reconstituted segment; lavage from the lacZ segment demonstrated a shift from polys to lymphocytes (Appendix K).

Cells in the lavage fluid were analyzed for recombinant gene expression using X-gal histochemistry for detection of lacZ or immunocytochemistry for

detection of CFTR. Analysis of fluid recovered three days after infusion revealed: a) In fluid recovered from the left upper lobe - lacZ expression in 5.2% of cells and CFTR expression in 0.3% of cells; and b) in fluid recovered from the right upper lobe - CFTR expression in 3.4% of cells and no cells expressing lacZ. Approximately 10^6 cells were recovered in the bronchial brushings, all of which have been placed in culture. Analysis of these cells has been restricted to SPQ assay for CFTR function.

IV.B.6. Is recombinant adenovirus likely to be expressed in the germline?

As shown in the data described above (see IV.B.2.), the intravenous administration of adenovirus in a quantity equivalent to that applied to the nasal surface did not lead to lacZ expression in the germline cells. Likewise, no evidence of germline expression was seen in male rabbits and monkeys (see IV.B.2.) receiving parenteral lacZ adenovirus.

IV.B.7. Recombination

Homologous recombination does occur within adenovirus serotypes. To minimize *in vivo* recombination, patients will be screened for immunity to Ad5 and administration of Ad5-CB-CFTR restricted to seropositive patients.

IV.B.8. Malignancy

Adenoviruses can cause tumors in newborn animals and can transform cells in tissue culture. The transforming function identified in tissue culture involves the E1A region. This region has been deleted in the proposed vector. No human tumor has been causally related to adenovirus (87-89).

IV.B.9. Integration

The data for Karlsson et al. (90,91) indicate that integration, if it does occur, is very low in cultured cells. No studies have been performed to test for integration in airway epithelia with a restricted life-span.

IV.B.10. Immune response

Adenoviral infections are associated with an immune response. This response is important for clearing adenoviral infections. Studies to determine the effects of this response on the feasibility of repetitive dosing of adenoviral vectors are underway.

V. HUMAN PROTOCOL

V.A. Experimental Design of Human Protocol

V.A.1. Introduction

The overall goal of the protocol will be to evaluate the safety and biological efficacy of CFTR gene transfer using recombinant adenoviruses administered to the nasal epithelium of patients with cystic fibrosis. The general design is a study of ascending dose for safety and for biological efficacy, with patients serving as their own controls for the treated side of the nose, and with contralateral sham-control nasal studies. At the completion of the study, we would expect to know 1) whether this recombinant adenovirus transfers the normal CFTR cDNA to nasal epithelial

cells to permit expression of normal CFTR mRNA transcripts and CFTR protein; 2) whether there is a local inflammatory response or the development of a systemic inflammatory response; 3) whether it corrects the abnormal ion transport phenotype seen in CF (i.e., raised nasal potential difference and the inability to secrete Cl^- in response to cAMP-mediated agonists); and 4) the duration of both responses.

V.A.2. Rationale

The nasal epithelium shares the ion transport abnormalities of the tracheobronchial epithelium in CF and demonstrates similar morphology and cell function as lower airway epithelium. We have performed extensive *in vivo* studies of ion transport in both nasal and lower respiratory epithelium of CF and normal subjects and used pharmacologic perturbation to explore the nature of the genetic defect in CF. In addition, the nasal epithelium is a site relatively free of bacterial infection, and is relatively accessible for administration of the recombinant adenoviral vector and for sampling epithelium for studies of inflammation and expression of mRNA and protein. It is also accessible for performing studies of biological efficacy [potential difference (PD)] measurements.

V.B. Patient selection

The initial study will be composed of 9 CF patients; each of three escalating doses of CFTR will be administered to three patients.

Inclusion criteria

1. Patients with the diagnosis of CF based on characteristic clinical disease and a sweat Cl^- test of $> 60 \text{ mEq/L}$.
2. Mild to moderate disease, i.e., $\text{FEV}_1 > 40\%$ predicted, (or $\text{FEV}_1 \geq 1 \text{ L}$), and stable pulmonary disease, i.e., no pulmonary exacerbations within the past 3 weeks.
3. Male and/or female subjects $\geq$ age 18 years.
4. Genotype: The "gold standard" would be homozygous ΔF508 patients. However, gene heterozygotes for ΔF508 are eligible if the patient has pancreatic insufficiency, i.e., does not have a mild mutation on the other allele, as are patients who have no ΔF508 mutation if their clinical disease is compatible with having two "severe" mutations, i.e., pancreatic exocrine insufficiency.
5. Seropositivity for antibody to adenovirus type 5. Seropositivity is very common ($> 70\%$) in the general population, and systemic immunity likely is desirable in this research to minimize any potential dissemination of the instilled virus.
6. No organ system disease (cardiac, hepatic, renal, endocrine) of a severity expected to limit an adequate systemic response to stress associated with experimental procedures. In general, this means normal organ function, except for biochemical abnormalities commonly seen with CF hepatic disease or glucose intolerance. Patients with CF-type insulin-requiring diabetes (not juvenile, ketotic-type diabetes) may be included if their insulin requirement is relatively low ($< 20 \text{ U NPH/day}$) and their diabetes is stable.

Exclusion criteria

1. Current unstable lung disease, i.e., worsening of pulmonary function or pulmonary symptoms of an "exacerbation" in the past three weeks.
2. Hypoxemia with a pO_2 of <60 mm Hg on room air.
3. Lack of adequate birth control, i.e., women may be included if they have practiced contraception for at least one month and have a negative pregnancy test.
4. Acute rhinosinus disease, including obstruction with nasal polyps, or recurrent sinusitis requiring repeated administration of antibiotics.
5. Systemic corticosteroids within the past three months, or other immunosuppressive medications.
6. Juvenile-type (insulin dependent and ketotic) diabetes mellitus.
7. Current use of DNase.
8. Chronic adenoviral shedding.
9. Participation in another therapeutic research study within 30 days.
10. Patients with known mild variants (R117H; R75Q), especially those with pancreatic exocrine sufficiency.
11. Inability to comply with the protocol.

V.C. Clinical evaluation of patients

Patients will be enrolled in the study at least four weeks before administration of the recombinant adenovirus and will be followed as outpatients at two week intervals until admission to the General Clinical Research Center (GCRC) for application of the recombinant virus. At the time of screening, patients will receive the evaluation outlined below, which will be repeated at the time of admission to the GCRC. Patients may also be evaluated with the following tests as appropriate for any change in clinical status.

1. Complete history and physical exam, including nasal endoscopy.
2. Genotyping for CF mutation.
3. Pulmonary function tests, including spirometry and arterial blood gases.
4. PA and lateral chest X-ray.
5. Electrocardiogram
6. Culture of sputum, nasal swab, throat swab, stool and urine sampling for wild type adenovirus.
7. Bacterial culture of sputum.
8. Nasal wash for inflammatory cell count.
9. Blood for CBC, including platelets and differential; Chem 18, including general chemistries and liver function tests; adenoviral antibody titers, including serotyping, and for viral culture.

10. Urine sample for analysis and bacterial and viral culture.
11. Measurement of transepithelial electric potential difference in the nasal epithelium.
12. Nasal brushings and biopsies as described below.

V.D. Clinical protocol

1. Patients will be admitted to the GCRC and placed in isolation in a hospital room with standard respiratory precautions. A respiratory isolation sign will be placed outside the door, and hand washing will be practiced upon entering and leaving the room. Visitors must wear gloves, gown, and mask, as per usual in reverse isolation procedures at the UNC Hospitals. If the patient leaves the room, a mask will be required.

2. Preparation of viral dose: The Ad5-CB-CFTR will be obtained as a purified and quality characterized virus from the University of Michigan Adenoviral Core Facility (J.M. Wilson, P.I.). The virus will arrive via overnight carrier. It will be opened and its contents directly placed in a syringe in Room 775 of Burnett-Womack. It will be instilled into the patient's nostril in the patient's room at the GCRC.

3. For application of the virus, the patient will be placed in a lateral recumbent position such that the recombinant adenovirus can be applied to the inferior turbinate and the solution will move to the dependent area of the inferior meatus (beneath the inferior turbinate). Topical lidocaine (2%) will be applied to the nasal mucosa immediately before application of virus. Under direct vision, the recombinant virus will be applied to the surface of the inferior turbinate utilizing a syringe pump, with application of approximately 2 ml being superfused over a period of 30 min.

4. For control studies, the patient will be repositioned into the opposite lateral recumbent position and vehicle (2 ml) perfused onto the contralateral nasal turbinate over 30 minutes.

5. Three different doses of Ad5-CB-CFTR will be used in a dose escalating fashion.

Patient	Volume	Titre	Dose
1-3	2 ml	10^8	2×10^8 pfu
4-6	2 ml	3×10^9	6×10^9 pfu
7-9	2 ml	10^{11}	2×10^{11} pfu

Assuming that the surface area (8 cm^2) of the nasal mucosa to be studied has 2×10^7 cells, a dose of 2×10^8 pfu's will correspond to a dose of ~ 10 pfu/cell; a dose of 6×10^9 pfus will correspond to a dose of 300 pfu/cell; and a dose of 2×10^{11} pfus will correspond to a dose of 10,000 pfus/cell. We will assess the results of each dose before escalating to the next dose.

V.E. Evaluation of adenoviral effect

Introduction. After the virus is instilled into the nasal epithelium, we estimate that the patient will be studied in the GCRC for 14 to 21 days. The patient will be confined to the GCRC room except as required for tests out of the room. During such times, patients will wear a mask to cover the nose and mouth, and strict respiratory and body fluid isolation will be accomplished. The patient will not be discharged from the hospital until two consecutive viral cultures are negative. The usual therapies for CF will continue through the study protocol.

V.E.1. To assess for safety:

V.E.1.a. Inflammation

V.E.1.a.(i) Patients will be monitored daily for symptoms of local or systemic inflammation. The site of application of the recombinant virus and control nostril will be compared by nasal endoscopy. Evidence of inflammation will be recorded.

V.E.1.a.(ii) The nasal epithelium will be biopsied for assessment of inflammatory infiltrate and for evaluation of cytopathic effects. This procedure will be performed by our ENT colleague, Dr. Pillsbury. The patients will be anesthetized with solution of topical anesthetic and epinephrine placed under direct vision. The region of the biopsy will be then infiltrated with anesthetic and epinephrine and 0.5 cm² of nasal mucosa will be resected by biopsy forceps with a cotton pledget placed at the biopsy site to effect hemostasis. The biopsy will be assessed for integrity of the epithelium and for evidence of inflammatory changes by light microscopy. Electron microscopy will also be performed to assess for cytopathology.

V.E.1.a.(iii) Blood/serum will be monitored serially for assessment of humoral antibody response to the recombinant virus and for any systemic response after the viral application. Blood and urine will be collected, and antibody titers to Ad5-CB-CFTR will be assessed by ELISA. Nasal lavages will be assessed for evidence of IgA antibody titers to Ad5-CB-CFTR. Blood and serum analyses will include CBC with white count and Chem 18 performed in UNC Hospital labs. The blood buffy coat, urine and stool will be cultured in a plaque-forming assay on 293 cells to assess for virus.

V.E.1.b. Shedding

Sequential swabs of the nose and the pharyngeal mucosa (and nasal lavages) and urine and stool samples will be undertaken to assess for viral shedding by culture and to assess inflammatory response by histology. Live recombinant virus will be tested for by plaque-forming assay on the 293 cell line. The virus is likely to be present for the first several days but disappear after a week. Evidence of inflammatory response will be assessed by counting inflammatory cells in the nasal wash specimen.

V.E.2. The assessment of efficacy will focus primarily on measurement of:

V.E.2.a. Transepithelial potential difference across the nasal epithelium.

Patients with CF have a raised PD, a low basal Cl^- permeability, and little response to cAMP-mediated agonists that stimulate Cl^- secretion across normal nasal epithelium. The abnormalities in CF nasal epithelium are well characterized *in vivo* in our laboratory, and correction of these basal abnormalities would suggest that recombinant virus has complemented the CF gene defect and corrected the ion transport abnormality. Measurement of nasal PD has been repetitively used in our lab without difficulty. It is a non-invasive test that can be performed serially without damage to the nasal epithelium. Briefly, the exploring electrode is a PE50 tubing that is perfused sequentially with either a Ringer solution or drug solutions to test for Cl^- secretion. The reference electrode is a 4% agar/Ringer solution in a 21 gauge needle that is placed in the subcutaneous space. Potential difference is measured between balanced calomel half-cell electrodes and using a high impedance voltmeter. This technique has been employed hundreds of times at UNC without complications.

V.E.2.b. Immunocytochemical detection of transduced CFTR

Efficacy will also be assessed in terms of evidence of CFTR protein production and localization at the apical membrane by immunocytochemistry using antibodies against CFTR. These will be performed on nasal epithelial specimens using a small cytology brush, nasal epithelia using a "scrape-biopsy" technique, and on standard nasal biopsy specimens. Nasal epithelial specimens will also be tested for the presence of mRNA by PCR techniques.

Flowsheet of Studies after Adenoviral Instillation

	Day 1	2	3	5	7	9	11	13
Nasal PD (same/contralateral)	+	+	+	+	+	+	+	+
Nasal swab/wash* (same/contralateral)	+	+	+	+	+	+	+	+
Pharyngeal swab (for virus)	+	+	+	+	+	+	+	+
Blood‡	+	+	+	+	+	+	+	+
Urine/stool (for virus)	+	+	+	+	+	+	+	+
Nasal scrape (same/contralateral)			+	+				+
Nasal Biopsy (same/contralateral)				+				

* Inflammatory cells (neutrophils); cytokines (IL8-IL1); virus

‡WBC and differential daily; anti-Ad5CFTR antibodies day 7 and 15;
Buffy coat for viral culture; Prothrombin time before biopsies.

After viral cultures are negative on two occasions and discharge from GCRC is anticipated, patients will have repeat chest X-ray, spirometry, arterial blood gas.

After discharge from the GCRC, patients will be followed on a weekly basis to monitor nasal PD (to test for duration of correction of bioelectric abnormalities) and to monitor nasal washes for evidence of inflammation until baseline status is re-established.

Interpretation of data:

These studies will provide information crucial to the safety of this recombinant virus, i.e., the possibility of local inflammatory response or systemic effects, and to whether this adenoviral vector can effectively correct the CF gene defect in the nasal epithelium, as indexed by changes in nasal potential difference and expression of CFTR mRNA and protein. It will provide information about the duration of any effect for correcting the gene defect. The study design employing escalating doses should provide information regarding dose-effect relationships for both efficacy and safety.

The importance of these initial studies in gene transfer is that they are critical to define the safety of these vectors and to provide compelling evidence that

gene transfer and correction of the defective CF-related airway epithelial cell function can be corrected. The design of the current study makes it highly likely that at least some of this information will be forthcoming. These results will play a key role in the design of future studies that address delivery of the gene to the tracheobronchial tree. A lack of success in correcting the CF cell dysfunction, or evidence of a substantial inflammatory response (particularly if persistent), will provide a clear impetus for pursuing the design and development of alternative vectors for gene therapy of CF. Alternatively, successful correction with little adverse effect will set the stage for a study to see if repetitive correction can be achieved.

V.F. Risk/benefit ratio for these studies:

The risks of this study are largely related to the possibility of (1) inflammatory and immunological responses that are of sufficient magnitude and duration to be of concern; (2) replication of the virus; (3) risk of environmental spread of the recombinant virus; and (4) risks associated with study procedures.

(1) Inflammation and immune response: Local inflammatory and/or cytopathic effects are possible at the site of application of the virus. These possibilities include pain, mucosal edema and inflammation, and bleeding, although it is unlikely that serious long-term effects will occur. We have attempted to limit these risks by the use of small doses of virus in a limited area of nasal epithelium and the use of dose-escalating study design. We have selected the nasal epithelium as the site of initial challenge (rather than the tracheobronchial tree) as an additional safety feature, i.e., this study design will not threaten respiratory status. Another type of risk relates to the possibility of these study subjects not being able to receive subsequent adenoviral-mediated gene therapy to the tracheobronchial tree, i.e., it is possible that additional administration of an adenoviral vector could generate an immune response that would prevent subsequent treatment in the lower respiratory tract with the adenoviral CFTR vector. Although possible, it seems that this would be more likely to occur in patients who are seronegative to the adenovirus type 5, or perhaps patients who have repeated exposures to the virus.

(2) Viral replication: Although the adenoviral vector has been rendered defective for replication by deletion of a segment of the E1 region, it is possible that the virus may have some ability to replicate in human airway epithelium. It is also possible that super-infection with a wild type adenovirus, or provision of the missing E1 and E3 sequences by a latent or residual adenovirus from an earlier infection, could complement the recombinant virus and provide replication competency. On balance, however, these possibilities seem unlikely to occur or to produce significant complications.

Another possibility is recombination of Ad5-CB-CFTR and a wild type adenovirus. The most likely combination event would occur between a segment of a wild type sequence 3' to the CFTR gene. This recombinant would form two viruses: one containing the CFTR minigene, but this would exceed the wild type viral genome size and (to date) we have not been able to make this virus; and the second viral recombinant would be a wild type virus missing the E3 sequences, a construct that should not be any more pathogenic than either parent virus. Second, the

likelihood of patients being infected with the wild type virus is limited by selection of only seropositive patients, and by treatment of patients who are isolated from exposure to wild type viruses. Finally, co-infection with the wild type virus would place the defective virus at a distinct advantage for replication, resulting eventually in a wild type viral infection that would be self-limiting.

(3) Containment of the recombinant virus: This is extremely important, and the risk that the virus could be transmitted to another person seems extraordinarily unlikely. Replication of the recombinant adenovirus is unlikely, and the risk of spread to other people has been minimized by isolation procedures, including hand washing and use of gowns, gloves, and masks. The small dose of virus further limits the potential risk of exposure to others. The patient will also not be removed from isolation until there are two consecutive nasal specimens that are negative for live virus. Finally, any environmental release would be most likely associated with a recombinant virus with no CFTR biological activity (see #2 above).

(4) Risks associated with the procedures:

- a) The procedure of recombinant virus and vehicle administration to the nasal epithelium should have no substantial risk, i.e., there may be some discomfort from the position required to instill the virus or some local nasal pain, which will be limited by topical anesthesia. Drug sensitivity (lidocaine) is a risk, but is rare, and patients will be well screened by history for allergy.
- b) Measurement of nasal PD is well tolerated and does not produce significant discomfort. There is a theoretical possibility of infection at the site of the subcutaneous needle insertion; this has not been observed in more than 10 years of using this technique at UNC.
- c) Nasal washes and pharyngeal swabs are modestly uncomfortable, but are associated with no significant long-term risk. These are regularly employed in research studies at UNC.
- d) Blood sampling produces the possibility of modest discomfort and bruising without substantial long-term risk.
- e) Biopsy of the nasal epithelium will be associated with pain and discomfort, although these will be addressed with local anesthesia. The subjects face a small risk of bleeding after the biopsy is completed, but this potential complication will be addressed by standard ENT practices.
- f) Pulmonary function testing includes arterial blood gases; this procedure is associated with the risk of bleeding and bruise formation, and is somewhat uncomfortable.
- g) These patients will be subjected to relative isolation in a hospital room for ~two weeks, and are likely to suffer some emotional distress related to this isolation. We will attempt to minimize this by providing television (including cable), and a VCR for movies and video games. We will also provide facilities for exercising (bicycle or

treadmill, etc.) during this time period, either in the patient's room or another approved isolation site.

Potential benefits:

Patients are not expected to benefit directly from this study. The correction of epithelial function in a small area of the nose is not likely to affect overall clinical outcome. However, the information generated from this study is likely to benefit the CF patient population at large. Information will be crucial for the design of studies for delivery of gene therapy to the lower airways, and the successful development of genetic approaches to the treatment of human disease has potential benefit to CF patients and greater mankind.

On balance, the risks to the study subjects would seem to be small in view of the overall safety of the recombinant vector, the general study design involving a dose-escalation, and the use of the nasal epithelium for the initial pilot studies. The risk/benefit ratio for this study would appear justified in terms of overall advances in medical knowledge for this particular patient population.

VI. PRODUCTION OF CFTR ADENOVIRUS

A critical part of the safety aspects of this protocol relates to the quality control and quality assurance associated with recombinant virus production. It is critical to demonstrate that the recombinant virus can be reproducibly manufactured in a way that the final product is of sufficient therapeutic quality and free of replication competent virus or other adventitious agents.

Virus production is performed in a BL2+ facility at the University at Michigan that is solely dedicated to production of recombinant adenoviruses. A schematic of the laboratory is provided in Appendix L. Within this laboratory there is a 4 ft laminar flow hood and double stack incubator that is dedicated to the production of Ad5-CB-CFTR. The detailed standard operating procedures for producing virus are described below as is the strategy for assuring quality of the final product.

VI.A. Strategy and Standard Operating Procedures

The overall strategy for the production of Ad5-CB-CFTR for therapy is summarized in Figure 24. A master cell bank (MCB) of 293 cells will be established that has been evaluated for performance, in terms of production of recombinant adenoviruses, and for the absence of other pathogenic contaminants. The MCB will be infected with our initial Ad5-CB-CFTR virus to generate a lysate. Virus will be purified and cryopreserved in aliquots. The lysate and purified seed lot will be subjected to safety testing as described in section VI.G. and VI.H.

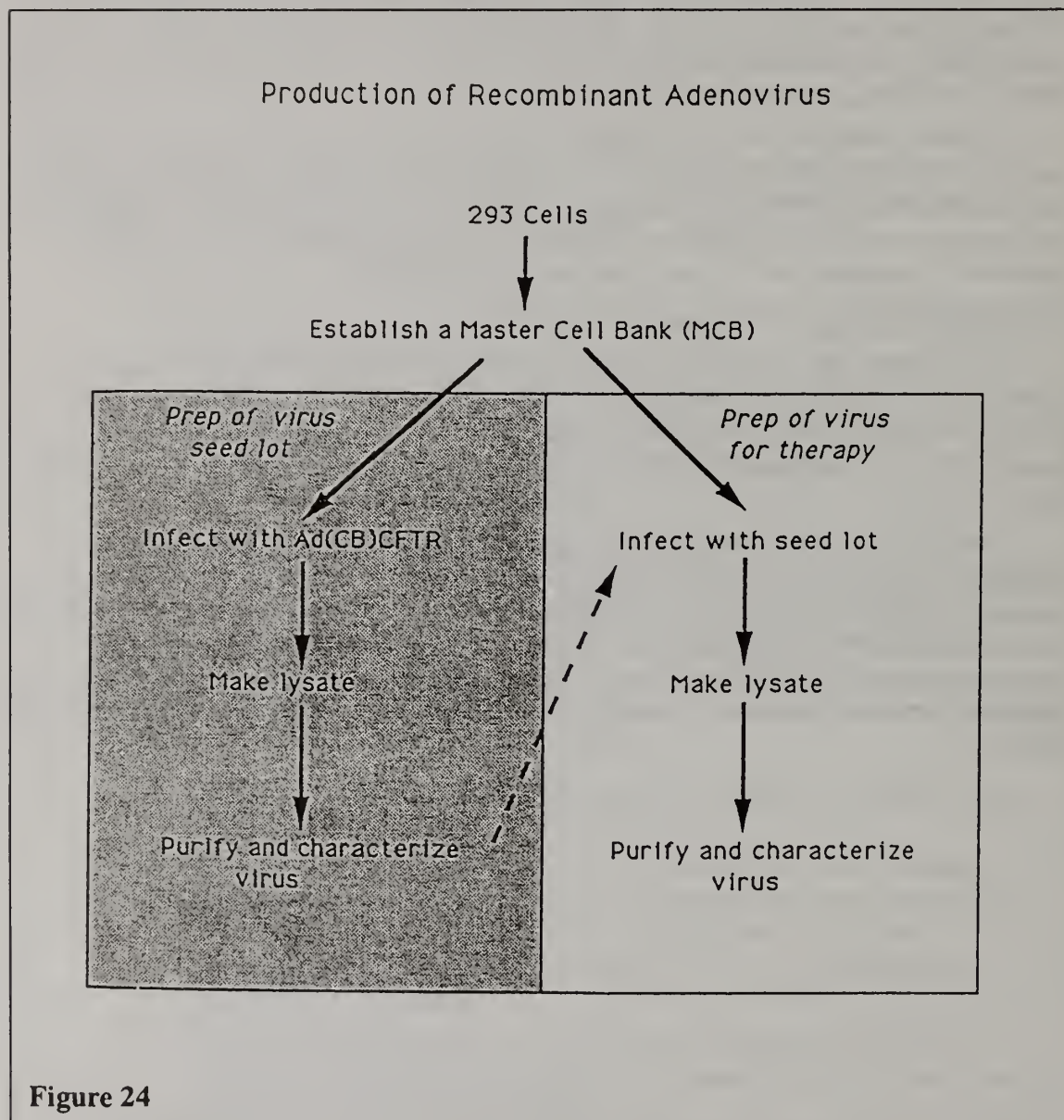


Figure 24

The MCB will be plated and infected with the virus seed lot. Lysates will be harvested from the infected cells and evaluated for sterility and mycoplasma testing. Virus will be purified from the lysate, cryopreserved, and lots will be evaluated as described in section VI.G. and VI.H.

VI.B. Production of Ad5-CB-CFTR

Thirty 150 mm plates of about 90% confluent 293 cells were infected with Ad5-CB-CFTR in 10 ml of DMEM/1% pen-strep with MOI of 10 for two hr., then 20 ml of DMEM/15% FBS/1% pen-strep was added. 36-40 hr post-infection, cells were harvested, pelleted, and resuspended in 10 mM Tris-Cl, pH 8.1. A viral lysate was generated by three cycles of freeze-thawing (ethanol-dry ice bath/37° C water bath). Cell debris was removed by centrifugation for 20 min at 3,000 rpm at 4° C, and washed once with 10 mM Tris-Cl, pH 8.1. Supernatants were pooled and loaded onto 20 ml of CsCl gradient made up of each volumes of 1.45 g/ml (density) and 1.20 g/ml CsCl in 10 mM Tris-Cl, pH 8.1. Following a two hr spin at 20,000 rpm at 4° C in a SW 28 rotor, the adenovirus band was removed by puncturing the tube with a needle, diluted with an equal volumes of 10 mM Tris-Cl, pH 8.1, and loaded onto a new gradient (8 ml). Following an overnight spin at 20,000 rpm at 4° C in a SW41 rotor, the viral band was removed and either stored at -20° C by diluting 1:5 into a glycerol/BSA solution (10 mM Tris-Cl, pH 8.1, 100 mM NaCl, 0.1% BSA, 50% glycerol) or purified by gel filtration through a Sephadex G50 column for immediate use. Viral yield was determined by OD₂₆₀ (Particles/ml = OD₂₆₀ × dilution × 10¹²/ml). Generally, a total of 3×10^{13} viral particles was achieved from 30 plates.

VI.B.1. Adenovirus seed lot

The adenovirus seed lot has been sent to a contract lab to be analyzed by numerous tests. The following summarizes those tests and their results.

Test	Result
Sterility	negative (final)
Mycoplasma	negative (preliminary)
Parvovirus B-19 hybridization	still on test
Electron Microscopy	still on test
AAV Hybridization	negative (preliminary)
<i>In vitro</i>	still on test

VI.C. Quality Assurance and Quality Control

A four-stage test program has been designed to assess the cell bank, product intermediate (cell lysate) and the purified product (virus). The 293 cells used to produce the adenovirus will be characterized prior to infection for possible microbial, adventitious viral, and select specific human viral contaminants. Testing of the adenovirus preparation used to infect the cells will include assays for microbial contaminants and adventitious virus. After expansion of the infected cells, the cell lysate will be evaluated for microbial contaminants. Product testing of the purified product for endotoxin, microbial contaminants, extraneous toxins and infectious adenovirus completes the test battery. Each step will be carefully tested for the presence of wild type adenovirus. The majority of tests will be performed by Microbiological Associates Inc. (MAI), an independent laboratory that has provided contract research and safety assessment for the pharmaceutical industry. A contract has been established with MAI for this project. 293 cells have been grown to

generate the Master Cell Bank (completed November 30, 1992), and certification tests have been started.

TABLE 1

<u>Process</u>	<u>Testing</u>
Master Cell Bank	Characterization of the cell bank
Infect cells with adenovirus	Adenovirus seed virus testing (adventitious contaminants)
Cell Lysate	Harvest Testing
Purified Product*- Ad5-CB-CFTR	Product Testing

*Bulk Harvest and/or Final Fill Material

VI.D. Characterization of Master Cell Bank (MCB)

The following tests will be performed on aliquots of the Master Cell Bank. these tests are based on the FDA's "points to consider" for use of mammalian cell lines. The MCB will be discarded if any test scores positive.

VI.D.1. Sterility Test (Protocol 1514.510 M.A., Inc.):

This test exceeds 21 CFR 610.12 in utilizing three different broth media and one agar medium for detection of both aerobic and anaerobic bacterial as well as fungal contaminants.

VI.D.2. Mycoplasma (Protocol 1514.102003 M.A., Inc.):

This protocol reflects the 1987 "Points to Consider" attachment and U.S. FDA expectations as to Mycoplasma testing. The study includes direct inoculation of test article on two types of agar and in the semi-solid broth medium. Broth inoculated with test article is subcultured at three intervals and incubated aerobically and anaerobically. In addition to the agar and broth cultivation, direct inoculation of the test article on Vero indicator cells and staining with Hoechst stain permits microscopic examination for the presence of Mycoplasma.

VI.D.3. Electron Microscopy (Protocol 1516.013 M.A., Inc.):

This protocol is designed to evaluate cell cultures for the presence of virus by examination of fixed cell pellets using transmission electron microscopy.

VI.D.4. *In Vitro* Virus Assay (Protocol 1514.003 M.A., Inc.):

A broad spectrum of viral contaminants are detected using an *In Vitro* Assay with sensitive indicator cells. Indicator cells, including the test article cells, MRC-5 cell line, and Vero cells. Test procedures include direct inoculation of test article as well as blind passage of supernatant fluids at 14 days. Indicator cells are observed

for cytopathic effect for the entire 28 day period. Hemagglutination and hemadsorption are performed twice during the assay.

VI.D.5. *In Vivo* Virus Assay (Protocol 1514.005002 M.A., Inc.):

This test includes the inoculation of adult mice, suckling mice, guinea pigs and embryonated hens' eggs via yolk sack and allantoic routes of inoculation. All animals are observed for clinical signs of viral infection. Suckling mice are sacrificed on day 14 for the preparation of a tissue homogenate which is inoculated into a new group of suckling mice. Specific Pathogen Free (SPF) eggs are examined for viability and the blind passage of fluids into new eggs is performed.

VI.D.6. Karyology Assay (Protocol 1516.018 M.A., Inc.):

This test is designed to confirm the species identity of cultured cells by means of isozyme and cytogenetic analysis.

VI.D.7. Tumorigenicity Assay (Protocol 1514.001 M.A., Inc.):

This study utilizes athymic nude mice which are inoculated subcutaneously with cells. Animals are observed for clinical signs, palpated bi-weekly, and submitted for histopathology evaluation of multiple tissues. Any animal which shows a regressing nodule is sacrificed and submitted for histopathological examination.

VI.D.8. EBV Probe Assay (Protocol 1516.104 M.A., Inc.):

The purpose of this study is to detect EBV DNA that may be present in the test article as determined by Southern hybridization using a labeled DNA probe. DNA is extracted from the test article cell pellet and blotted onto membranes. Blotted membranes are hybridized to a labeled EBV probe and detected by autoradiography. DNA from Namalwa cells are included as a positive control.

VI.D.9. Cytomegalovirus (Protocol 1514.030 M.A., Inc.):

The test and control articles are directly inoculated onto cell cultures (indicator cells) and examined for 42 days for the appearance of cytopathic effect. Additionally, cells are fixed and examined using immunofluorescent techniques.

VI.D.10. HIV Co-cultivation Assay (Protocol 1516.015001 M.A., Inc.):

This procedure is designed to detect small amounts of retrovirus that may be present in the test article. To amplify any virus present, selected Peripheral Blood Lymphocytes (PBL) cells are infected, mixed with test article, passaged and analyzed for HIV by observing for cytopathic effect (CPE) and production of viral antigens by antigen capture ELISA.

VI.D.11. Bovine Virus Assay (Protocol 1514.032001 M.A., Inc.):

This study is designed to detect bovine viruses such as Bovine Viral Diarrhea Virus (BVD), Infectious Bovine Rhinotracheitis Virus (IBR), Parainfluenza 3 (PI 3), Bovine Adenovirus (BA), or Bovine Parvovirus (BP) using sensitive indicator cells and immunofluorescence and cytopathic effect (CPE) as endpoints.

VI.D.12. Hepatitis B (Protocol 1516.040 M.A., Inc.):

This procedure is designed to detect small amounts of Hepatitis B surface antigen that may be present in the test article which would be detected by a third generation ELISA assay.

VI.D.13. Porcine Parvovirus (Protocol 1514.033004 M.A., Inc.):

This assay is designed to detect Porcine Parvovirus (PPV) in cell cultures. Test and control articles are analyzed by inoculation of indicator cell cultures and examined for at least 14 days for the presence of CPE. Cultures are also tested for the presence of specific viral antigens using fluorescent antibody techniques.

VI.D.14. Adeno-Associated Virus Hybridization (Protocol 1516.104016 M.A., Inc.):

This assay detects adeno-associated virus DNA present in the test article as determined by Southern hybridization using a ^{32}P labeled DNA probe.

VI.D.15. Parvovirus B-19 Hybridization (Protocol 1516.104017 M.A., Inc.):

This protocol is designed to detect parvovirus B-19 DNA that may be present using Southern hybridization with a ^{32}P labeled DNA probe.

VI.D.16. Replication Competent-Adenovirus Assay (Protocol 1514.031002 M.A., Inc.):

This assay is designed to detect the presence of human adenovirus in the test article. Indicator cell cultures are inoculated and observed periodically for CPE, a passage is performed for enhancement.

VI.E. Characterization of adenovirus seed Lot

Each seed lot will be prepared, cryopreserved and aliquots evaluated according to the following criteria:

Mycoplasma (M.A., Inc.)-see above

Sterility (M.A., Inc.)-see above

In vitro virus (M.A., Inc.)-see above

Adeno-associated virus (M.A., Inc.)-see above

Parvovirus B-19 hybridization (M.A., Inc.)-see above

Electron microscopy (M.A., Inc.)-see above

Replication competent Ad5-please see description of assays in section

VI.H.

Presence of functional Ad5-CB-CFTR-see description of assay in section VI.H.

VI.F. Characterization of cell lysate

Each preparation of cell lysate will be pooled and aliquots will be evaluated for Mycoplasma and sterility (see above for descriptions) before virus is purified. Any lysate demonstrating contamination will be discarded.

VI.G. Characterization of purified lots of Ad5-CB-CFTR for therapy

Aliquots of each lot will be subjected to the following tests before use:

Sterility (M.A., Inc.)-see above

General Safety (Protocol 1514.509 M.A., Inc.):

This assay is designed to detect extraneous toxic contaminants. Guinea pigs and mice are inoculated with test material and observed for overt signs of ill-health or unusual response.

Limulus Amebocyte Lysate (LAL) (Protocol SPGT332070 M.A., Inc.):

The purpose of this study is to detect and quantitatively determine the gram negative bacterial endotoxin level of a test article or extract using the Limulus Amebocyte Lysate (LAL) gel-clot method for testing.

Replication Competent Ad5-please see description of assays in section VI.H.

Presence and quantity of functional Ad5-CB-CFTR-see description of assay in section VI.H.

VI.H. Assays for functional CFTR virus and contaminating wild type Ad5 virus

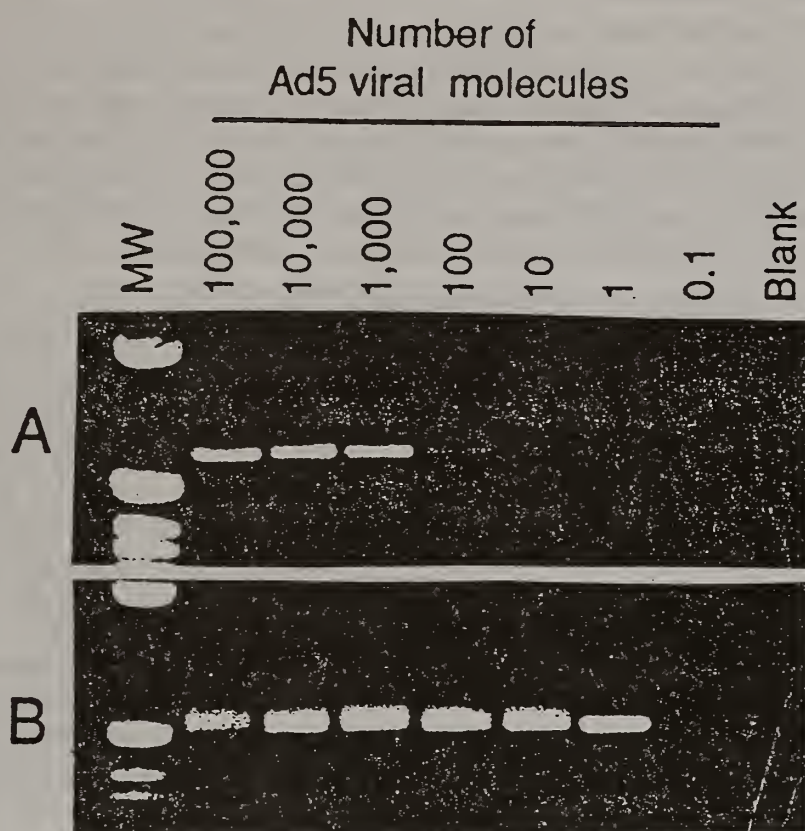
The final preparation of purified Ad5-CB-CFTR virus will be evaluated for the presence and concentration of virions capable of correcting the CF defect. The total particle number will be determined by measurement of the absorbance at 260 nm. An aliquot of the virus preparation will be diluted serially and used to infect both 293 cells and the cell line CFPAC which was derived from a pancreatic adenocarcinoma of a patient with cystic fibrosis. The infected 293 cells will be evaluated for E1 transcomplementing virus using the previously described plaque forming assay. The infected CFPAC cells will be evaluated for CFTR protein expression by immunocytochemistry and correction of cAMP mediated chloride transport using SPQ assay. Descriptions of these assays are provided in section IV.A.2.B.(iii).

Several experiments will be performed to evaluate the virus preparations for contamination with wild type adenoviruses. The presence of wild type adenoviruses in the 293 cell line will be evaluated by M.A., Inc. using a standard assay in which a

variety of indicator cell lines are exposed to 293 supernatants, passaged, and inspected for cytopathic effects.

Detection and quantitation of low level wild type adenovirus in high titer preparations of the recombinant Ad5-CB-CFTR virus is confounded by the observation that E1 deleted viruses will cause cytopathic effects (CPE) in non E1 expressing cells, such as HeLa cells infected at high MOI's. Mechanism(s) for this relate to the direct toxic effect of high concentrations of viral proteins such as fiber and the possibility of low level viral replication of E1 deleted virus at high MOI. We find it necessary to dilute the concentrated stock at least 100 to 1000 fold to avoid CPE in most indicator cell lines. Two types of assays will be performed. A typical virus preparation contains 5×10^{12} particles/ml based on OD at 260 nm and $\sim 10^{11}$ functional virions/ml based on pfu or assays that measure CFTR protein expression (immunocytochemistry or SPQ functional analyses). The first assay is based on the ability of wild type Ad (but not E1 deleted Ad) to replicate in non-E1 expressing cell lines. An aliquot of each preparation representing 5% of the total will be diluted and exposed to a variety of indicator cell lines. The cells are passaged for 2 weeks to allow spread of wild type virus and subsequently examined for CPE. Specifically, for each ml of stock, 5 μ l is removed, diluted and exposed to 10 x 15 cm plates of confluent indicator cells. Preliminary results with purified preps of recombinant viruses have failed to demonstrate CPE under these conditions. The sensitivity of the assay is currently being defined in reconstitution experiments.

Another assay has been developed to detect and quantify wild type Ad5 in the stocks of recombinant virus. The potential sources of wild type Ad5 are contamination from the initial transfection or recombination with Ad5 sequences originally transfected into the 293 cells. The assay is based on PCR detection of E1 sequences in DNA isolated from the stock of recombinant virus. Figure 25 shows an agarose gel of PCR products in which 125 ng of total cellular DNA was supplemented with varying amounts of wild type Ad viral DNA (from 100,000 to 0.1 copies of Ad5 per reaction). Amplification for 35 cycles afforded a sensitivity equal to 100 viral molecules/reaction (panel A). However, when the initial reaction products were used as a template for another 35 cycles of amplification using nested primers, the sensitivity was improved to 1 viral molecule/reaction (panel B). DNA will be isolated from a five percent of each viral stock and subjected to the PCR analysis. Five percent of a 2 ml viral stock should yield 2-20 mg of DNA which can be analyzed in 4 to 40 reaction tubes.



PCR assay for detection of wild type Ad5 virus particles. Total cellular DNA (125 ng) was supplemented with varying quantities of Ad5 DNA (0.1 to 100,000 Ad5 molecules/tube). These mixtures were subjected to 35 rounds of amplification with primers to E1a (5'-501 to 524, and 3'-1075 to 1099). Panel A shows agarose gel analysis of these reactions. An aliquot (2 μ l) of the first round reactions were amplified another 35 cycles with nested primers to E1a (5'-527 to 548 bp and 3'-1029 to 1053 bp). Agarose gel analysis of the second round PCR is shown in Panel B.

Figure 25. PCR assay for wild-type adenovirus contamination.

VII. OVERALL RISK/BENEFIT

VII.A. Risks to human subjects

VII.A.1. Blood draws

Localized hematoma at the puncture site- appropriate pressure will be applied to the puncture site to prevent this common complication. Vaso-vagal faint- patients will be asked to report any symptoms of lightheadedness or circumoral tingling which may precede a vaso-vagal episode. those individuals experiencing such symptoms will be kept in a recumbent position until this temporary reaction abates. If the above precautions are taken, the potential risk to blood donors is negligible.

VII.A.2. Nasal PD

Nasal PD studies have been performed ~1,000 times at UNC over the past decade without significant incident. There is the possibility of infection at the site of subcutaneous reference needle insertion; this has not occurred over the past 10 years at UNC. Drug sensitivity to amiloride or isoproterenol is a risk, but we have not observed such an event. We have had several patients suffer vaso-vagal reactions during the procedure; these are handled by placing the patient in a recumbent position, and all have recovered with no adverse effect.

VII.A.3. Ectopic or overexpression of CFTR

We have performed a series of studies to address these issues. The most compelling experiments involved the generation of transgenic mice that had been programmed to express high levels of human CFTR in the airway. One set of animals expressed CFTR from a promoter of the SPC gene that is very active in the distal airway including the alveolar cells and the distal bronchiolar cells. Another set of animals expressed CFTR from a promoter of the CC10 gene that is very active in most of the cells of the proximal airway. All of the transgenic lines expressed high levels of human CFTR protein (which is active in mouse cells) in the appropriate cells and none of them demonstrated pathology or clinical abnormalities. This suggests that expression of recombinant CFTR in the wrong cell or unregulated, overexpression in the correct cell will not be deleterious. These studies are complemented by studies in human airway cell lines that indicate no toxic effects of overexpression of CFTR on growth characteristics and cell volume regulation.

VII.A.4. Replication of the virus and expression of viral proteins

Ad5-CB-CFTR has been engineered in a way that the region responsible for activating its transcriptional units, called E1, has been deleted. In principle, therefore, the recombinant genome should not replicate or express viral proteins in the recipient cell. In practice, certain cell types under special conditions can transactivate the viral genes using endogenous cellular factors.

We have established a model of the CF airway that is based on the growth of human airway epithelial xenografts in nu/nu mice to better address these important issues in a relevant biological setting. This model was used to evaluate the possibility of viral protein expression and replication of the E1 deleted CFTR virus in the context of a human CF epithelium. These studies failed to detect viral protein expression. A low level of ongoing replication of the virus in human xenografts that were stably expressing the recombinant gene has been detected. Thus, low level replication can occur in human airway epithelia housed in an immunocompromised host (the nu/nu mouse). The possibility of low level replication was subsequently tested in a species closely related to humans (baboon). In the baboon, no replication of virus was detected in protocols that span the viral doses employed in this study. However, the baboon may be relatively incompetent for adenovirus infection, and a series of studies are underway in a species that is clearly competent for adenovirus 5 infection, i.e., the cotton rat. Low level replication is not seen with wild type adenovirus in immunocompetent hosts, and we

believe it unlikely that replication of Ad5-CB-CFTR will occur in immunocompetent hosts. We will limit our trials to immunocompetent CF patients and include steroid use as an exclusion criterion.

It is important to consider the consequences of the unexpected dissemination of the recombinant virus or a replication competent derivative. Previous experience with adenoviruses in humans suggests that they are relatively benign. Adenoviruses are endemic in the population causing a variety of self limited infections. Infection with Ad5 causes mild respiratory infections characterized by coryza, pharyngitis, fever, malaise, and tonsillitis. The relative safety of adenoviruses was evaluated in a series of experiments, performed in the late 1960's and early 1970's, in which volunteers were exposed via the respiratory tract to wild type forms of adenoviruses including the serotype used in this study (Ad5). For example, in a study by Couch et al. (92), normal adult volunteers were immunized to Ad5 capsid antigens and subsequently exposed to an inoculum of live Ad5 into the respiratory tract. Ten of 19 individuals who received a placebo immunization and subsequently live Ad5 had a self limited febrile illness characterized by fever and moderate pharyngitis. No significant untoward effects were noted.

An additional consideration relates to the malignant potential of these gene transfer reagents. In contrast to retroviruses which integrate into the genome, adenoviruses are maintained in the genome as extrachromosomal elements which theoretically cannot cause a secondary malignancy due to insertional mutagenesis. In support of this is the fact that no human malignancy has been directly linked to adenoviruses.

Psychological and social implications. It is likely that the initiation of these experiments will be associated with tremendous attention by the media. We will follow a strict code of confidentiality as mandated by the RAC and inform the patients of this potential problem.

VII.B. Risks to others

The only potential risk to others is the possibility that the recombinant virus could be transmitted to another individual, take up residence, and cause disease. We believe this is highly unlikely for a variety of reasons.

One very important aspect of this scenario relates to the likelihood that transmission of Ad5-CB-CFTR will cause disease. We obviously do not think this is likely based on the fact that we are proposing to use it therapeutically. Toxicity could be due to one of two mechanisms: expression of CFTR or pathology due to the inherent properties of Ad5. As we argued above, unregulated overexpression of CFTR does not appear to be toxic. Furthermore, pathology of the recombinant virus due to its properties as an infectious virus is unlikely because all patients will have had previous immunity to the virus and the recombinant should have a tremendous growth disadvantage due to the deletion of E1.

Another possibility is that the Ad5-CB-CFTR will undergo a recombination with homologous wild type Ad to form a more toxic structure. As demonstrated below the most likely recombination will be between the large stretch of homology 3' to the CFTR minigene. This will form two recombinants: one containing the CFTR minigene with E3 sequences (this exceeds the wild type genome size; our attempts to actually make this virus have failed because it cannot be packaged) and a wild-type hybrid genome missing E3 sequences (there is no theoretical reason to expect this to be more pathogenic than either parent).

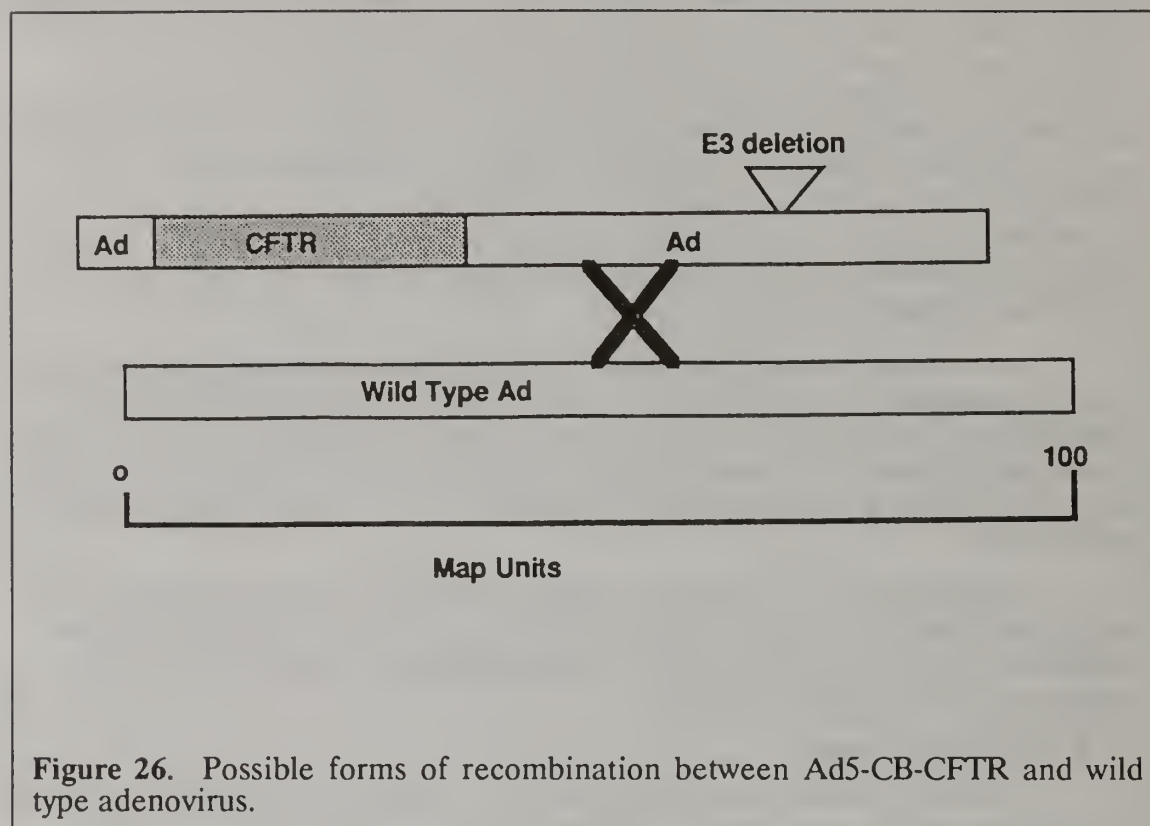


Figure 26. Possible forms of recombination between Ad5-CB-CFTR and wild type adenovirus.

The next consideration relates to the probability that the Ad5-CB-CFTR virus can replicate, spread and be transmitted. We believe that this will be unlikely based on our animal data. In order to assure that this does not occur we will keep the patients in isolation for a period of at least 10 days following the treatment. The patient's nasal swabs and sputum will be evaluated for Ad5-CB-CFTR sequences before discharge. If there is evidence of ongoing spread the patient will be asked to stay in the hospital for additional days. We think this will be extremely unlikely. The other possible situation that could lead to spread is if the patient becomes superinfected with a homologous wild type virus which mobilizes the recombinant genome. It is impossible for this to occur through the generation of a replication competent virus containing the minigene due to size limitations of packaging. The spread of the genome would have to occur by adenoviral functions provided in trans. We think this is highly unlikely and should not prevent the study. In order to address this potential concern we will work to be particularly cognizant of signs and symptoms characteristic of adenoviral infections and will continue to survey nasal swabs for Ad5-CB-CFTR sequences using the PCR assay.

VII.C. Potential Benefits

This is a phase I study so there will be no actual benefits to the patients involved in the experimental protocol. This study could have enormous benefit to the larger community of CF patients for a variety of reasons. Despite tremendous investment of resources by the pharmaceutical industry, management of the pulmonary complications of CF is inadequate; patients typically succumb to these complications by the age of thirty. The information gained in this study will provide information that is critical to the eventual development of effective treatments of the lung disease of CF by gene therapy. We hope to evaluate the overall safety of this approach as well as to assess the feasibility of obtaining genetic reconstitution that is therapeutic and stable. We do not believe that this trial will actually be therapeutic to the patient because the therapy will be administered to the nasal cavity.

We consider this an extremely important study with respect to evaluating the potential use of recombinant adenoviruses in the treatment of cystic fibrosis. We would consider this a successful experiment if we can answer the critical questions addressing the safety and efficacy of this approach to gene therapy in cystic fibrosis. However, based on our extensive work in animal models we believe there is a reasonable chance that the study will show recombinant adenoviruses are capable of safely transferring recombinant CFTR genes to the cystic fibrosis nasal epithelium and that the genes function for a prolonged period of time.

An important barrier to gene transfer into the surface epithelium of the airway is that the cells are relatively quiescent with approximately 0.1% of the cells going through the cell cycle at any one time. For this reason, approaches to gene therapy which depend on integration of the transgene into the recipient cell's chromosome, such as recombinant retroviruses, likely will not be effective in adults. Recombinant adenoviruses have the capability of transducing genes into nondividing cells and maintaining the transgene as an extrachromosomal element. We have used the human xenograft system to evaluate the stability of adenoviral mediated recombinant gene expression. We have found that recombinant adenoviruses are capable of efficiently transferring genes into nondividing fully differentiated cells of the surface epithelium and that the recombinant gene expression is stable for the longevity of the grafts which is approximately 4 weeks. Presuming the viral genome is stable in the cell, the persistence of recombinant gene expression becomes dependent on the lifespan of the cell. As described above the cells of the surface epithelium have a relatively long life span and xenograft experiments have demonstrated gene transfer into all cell types that line the lumen of the airway.

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Study number 92-MED-290

CONSENT TO PARTICIPATE IN A RESEARCH STUDY

TITLE OF STUDY: Gene Therapy for Cystic Fibrosis using E1 Deleted
Adenovirus: A Phase I Trial in the Nasal Cavity

Principal Investigators: Michael R. Knowles, M.D., Richard C. Boucher, M.D.

Co-Investigator: Larry G. Johnson, M.D.

I have been asked to take part in a research study under the direction of Michael R. Knowles, M.D. and Richard C. Boucher, M.D. I understand that I will be one of approximately 9 subjects in this Phase I study, which is a pilot (first time) study.

Purpose: I understand that this research study is designed to test whether it is possible to transfer safely a normal CF gene into the nasal cells of CF patients and correct the abnormal cell function that is associated with the genetic defect in CF. This method of treating patients with genetic diseases has been termed "gene therapy", and the ultimate goal of this initial study of gene therapy is to develop a better treatment for CF. The vehicle that will be used to carry the normal CF gene into the nasal cells will be a "cold" virus (adenovirus) that has been modified so that it should not multiply and spread throughout the body. This study will have two goals. The first goal is to determine if gene therapy can be accomplished safely in the nose, and thereby allow the possibility of later attempting treatment in the lungs. The second is to determine whether it is possible to correct the ion transport abnormalities in CF airway cells that are responsible for thick mucus. I understand that this study is not designed for treatment of the lungs, and that I am unlikely to get any medical benefit from this nose study of adenoviral gene therapy.

Duration: I understand that my most active participation in this study will last for approximately four months. I will be screened for this study at least four weeks before gene therapy is undertaken, and re-evaluated approximately two weeks before hospital admission. I will be in the hospital [General Clinical Research Center (GCRC)] for approximately 1 week before gene therapy, and for approximately two (possibly three or four) weeks after gene therapy. I will be monitored for several weeks after discharge from the hospital on a weekly basis, and then for two- to four-week intervals for a total of three months, and then at the end of a year. The initial (screening) outpatient visit will last most of a day, and the other outpatient visits will last several hours.

Procedures: I understand that during this study, the following will occur:

- 1) The study design will involve evaluation at least four weeks before gene therapy is initiated, and admission to a hospital room on the GCRC in respiratory isolation for one week for re-evaluation before gene therapy. Following gene therapy, I will remain in respiratory isolation in the GCRC for two (or up to four) weeks (see below), during which time the feasibility and safety of this method of gene therapy will be tested.
- 2) The adenovirus containing the normal CF gene is an experimental medication. The virus will be administered into the nose by slow perfusion through a small tube for 30 minutes. This will be accomplished while I am lying on my side so that the solution can be placed into the nostril under direct vision. A control solution (not containing virus) will be placed into the opposite nostril to allow comparison of nasal cell function before and after virus, and as compared to the side not receiving gene therapy. A numbing medicine (lidocaine) will be applied inside the nose before putting in the virus.
- 3) Nasal potential difference, i.e., "electrical activity" will be measured before and after virus to see if the abnormalities in CF nasal cells are corrected after gene therapy. This test is similar to the electrocardiogram, where electrical signals can be measured but not felt in the body. The electrical activity will be measured by inserting a needle under the skin of the forearm, and touching the inner surface of the nose with a small catheter. The electrical response to nasal addition of drugs will also be measured, including amiloride and isoproterenol (approved for human use). Amiloride is normally given for heart and renal disease. Isoproterenol is one of the original inhaled asthma drugs. This study will be performed approximately 10 times, beginning at the time of initial screening, and throughout the follow-up visits.
- 4) Nasal washes and throat swabs will be performed to test for inflammatory cells and presence of the virus. The nasal wash is performed by instilling one teaspoonful of salt water into the nose, tilting the head back for approximately 10 seconds, and collecting the expelled liquid when the head is tilted forward. The throat swab is performed with a cotton tipped swab applied to the back of the throat. These will be performed approximately 10-12 times from screening through the follow-up visits.

- 5) Blood will be drawn (about 2 tablespoonfuls) at the time of screening and through the hospitalization, and at follow-up visits. This will be performed approximately 10-12 times. The total volume of blood drawn during the study will be less than 1 unit (pint).
- 6) A small brush or plastic instrument for "scrape biopsy" of nasal cells will be inserted into the nose to collect cells to test for evidence of inflammation or virus. This procedure may be slightly painful, but numbing medicine (lidocaine) can be used, and symptoms typically last for less than an hour.
- 7) A nasal biopsy will be performed on one occasion by an ear, nose, and throat doctor to identify evidence of inflammation, and presence of the normal CF gene protein. This will be done using local anesthetic, and is usually associated with some local pain, nasal discharge, and/or minor bleeding.

All of the procedures and tests will be solely for this research study. However, many of the procedures that are performed (throat swab, blood drawing, urine collection) are standard medical procedures that are performed at UNC Hospitals.

Special considerations for the research subjects include:

- 1) Gene therapy is a new form of treatment, and the long-term outcome is not known. My agreement to participate in this study means that I recognize the need for careful follow-up. This will involve evaluation for up to a year with a medical history, physical examination, chest X-ray, and routine blood tests.
- 2) In the case of an unexpected death (such as from a car accident), a post-mortem evaluation would be valuable to search for any effects of gene therapy. My participation means that I would tend to favor an autopsy should such an occurrence take place. I will notify my family of my opinion, but I understand that this Consent Form does not bind my family to agree to an autopsy.
- 3) I will not be identified in reports about this study, but the public interest in gene therapy may lead the media to attempt to identify who I am. The researchers will do everything possible to protect my privacy, but I may be identified by other means and subject to frequent requests for interviews and my privacy may be reduced.
- 4) The study will involve three different doses of virus. The lower dose of virus will be completed in three patients before testing the mid-level dose, and the mid-level dose will be completed in three patients before testing the higher level dose (three patients). I understand that the chance of side-effects with each dose cannot be clearly predicted, although traditional concepts suggest that the higher doses may be more likely associated with side-effects.
- 5) After addition of the virus into the nose, I will be required to remain in the hospital in respiratory isolation for approximately two (or up to four) weeks. In part, this is for the public welfare to make certain that the recombinant virus is not spread around to the general public. I understand that if I attempt to leave

the hospital before the doctors have demonstrated that I have no virus in my nose or body, the researchers have a responsibility (and legal right) to keep me for at least 72 hours against my will until review of the situation takes place.

Several physicians will be responsible for my welfare during this study, i.e., Michael Knowles, M.D., Richard Boucher, M.D. and Larry Johnson, M.D.

Exclusions: I understand that I should not participate in this study if any of the following apply to me:

- 1) Worsening of pulmonary function or "exacerbation" in the past three weeks.
- 2) Lack of adequate birth control, i.e., men must be demonstrated to be infertile (azoospermic) and women must have practiced standard contraception (condoms, IUDs, birth control pills, or abstinence) for at least one month and have a negative pregnancy test.
- 3) Systemic steroids (cortisone) must not have been used within the past three months.
- 4) No participation in other therapeutic research studies within the past month, for example, the use of DNase.
- 5) Patients with known mild CF genetic mutations, specifically those with normal pancreatic digestive function.
- 6) Patients whose blood does not have antibodies to adenovirus type 5; a lack of "seropositivity" should exclude me from participating.
- 7) Patients without adequate organ system function (cardiac, hepatic, renal, endocrine), so that complications would not produce clinical difficulties.

Risks and Discomforts: I understand that this study might involve the following risks and/or discomforts:

- 1) Gene therapy is experimental, and several complications are possible.
 - a) Although the virus that is being used has been modified so that it cannot reproduce itself, there is a slight possibility that something would occur during the study that would allow the virus to multiply. The results of this event are not fully known, although it is likely that the virus would be killed after it has been there for several days to weeks, since my body has immunity to the adenovirus type 5.
 - b) The virus could damage nasal cells, or the inflammatory response could cause local inflammation in the nose. It is possible, although highly unlikely, that this could cause permanent damage. It would likely cause local pain or inflammation in the nose that would resemble a "cold", or could cause sinusitis.
 - c) It is possible that this virus could spread to others in the surrounding environment, although this is unlikely. However, if signs are present that this is occurring, or could occur, i.e., live viruses in nasal washes, I will be asked to remain in the hospital in respiratory isolation for up to four weeks after virus is placed in the nose.

- d) It is possible that participation in this study could cause my immune system to respond against the altered virus such that subsequent treatments with the altered virus would not be possible, or that an allergic reaction may be developed. That would mean that gene therapy would not be possible with this type of adenovirus.
 - e) The gene therapy may cause more than a normal amount of the normal CF protein to be produced in the nasal cells, and it is conceivable that this could be harmful or produce an allergic reaction. This does not occur in animals, even when many hundred times normal production is present; however, this has not been tested in humans.
- 2) Several procedures are likely to be associated with transient discomfort, including blood drawing for blood counts or arterial blood gas, measurement of nasal electrical activity, brushing or biopsies of the nasal epithelium, and respiratory isolation. Each of these procedures or situations are addressed with interventions, such as numbing medicine, to minimize these discomforts.
 - 3) The altered adenovirus could be dangerous to a fetus if I am pregnant. Pregnancy and breast-feeding excludes participation and birth control will be required. If a pregnancy accidentally occurs, the medical researchers should be notified immediately.
 - 4) During the study, I can have the usual problems of CF patients, including infection of my lungs. If I should develop a CF-related illness, the doctors would initiate appropriate treatment for that complication. If the study protocol is not felt to be responsible for provoking such a complication, usual methods of payment for CF treatment should be sought.
 - 5) I will have two or three chest X-rays during the course of this study; the dose of radiation is small.

Despite our best efforts, it is possible that unforeseen risks and/or discomforts may occur. We will do as careful a study as possible, but it must be recognized that unexpected events or complications could occur.

Benefits: I understand that the benefits of participating in this study relate to improved understanding of the possibilities for gene therapy in CF. I am unlikely to benefit directly from the nose study of adenoviral gene therapy. Overall, the greater benefits of this study relate to better understanding of modified viruses for delivering gene therapy, and for designing future studies, which might involve giving gene therapy to the airways of the lung.

Alternatives: I understand that there are standard medical therapies available if I choose not to participate in this study, and that this study is not likely to change my requirement for standard medical therapy for CF.

New Findings: I understand that I will be given any new information gained during the course of the study that might affect my willingness to continue my participation.

Confidentiality: I understand that every effort will be taken to protect my identity as a participant in this study. However, there is no guarantee that the information cannot be obtained by legal process or court order. I will not be identified in any report or publication of this study or its results. The FDA may review all the records of this study, and perhaps report it to other regulatory agencies.

Financial Costs of the Research: I understand that the costs of this research will be borne by monies from the N.I.H. and the CF Foundation. I will not be charged for any tests or admissions to the GCRC if done solely for research purposes. If I have a CF-related problem, that develops during this study, I understand that I would be responsible for the cost of treatment, just as I would be if I were not in the research study.

Compensation in Case of Injury: I understand that in the event of physical injury resulting directly from the research procedures, financial compensation cannot be provided. All forms of medical diagnosis and treatment, whether routine or experimental, involve some risk of injury. In spite of all precautions, I might develop medical complications from participating in this study. If such complications arise, the researchers will assist me in obtaining appropriate medical treatment but the University of North Carolina at Chapel Hill does not provide financial assistance for medical or other costs. I understand that I do not waive any liability rights for personal injury by signing this form.

Payments to Participants: I understand that I will receive \$75.00 for each day in the hospital and \$75.00 for each day of screening and follow-up visits for up to one year to compensate for the time and inconvenience of participation. In addition, travel expenses will be compensated to and from UNCH at the rate of \$.25/mile, and parking expenses. If I am withdrawn from the study, I will receive payment for completed visits and hospitalization days as described above.

Right to Refuse or to Withdraw from the Study: I understand that my participation is voluntary and that I may refuse to participate, or may discontinue my participation at any time without penalty, or jeopardizing my continuing medical care at this institution, or losing benefits that I would otherwise be entitled to. However, after the virus is placed in the nose, I also understand that freedom to discontinue participation in study procedures does not mean that I am free to leave the hospital (respiratory isolation) until the virus is not present, or the local Health Department officials (after discussions with the UNC Biosafety Committee, the Human Rights Committee, and the Principal Investigators) have approved my leaving the hospital.

I also understand that Drs. Michael Knowles, Richard Boucher, or Larry Johnson has the right to stop my participation in the study at any time. This could be because I have had an unexpected reaction, or have failed to follow instructions, or because the entire study has been stopped.

Offer to Answer Questions: I have the opportunity to ask, and have had answered, all my questions about this research. If I have other questions, or if a research-related injury occurs, I will call Michael Knowles, M.D., Richard Boucher, M.D., or Larry Johnson, M.D. at (919) 966-1077.

I understand that this project has been approved by the Committee on the Protection of the Rights of Human Subjects at The University of North Carolina at Chapel Hill. If I believe that there is any infringement upon my rights, I may contact the Chairman of the Committee, Ernest N. Kraybill, M.D. at (919) 966-1344.

I have read the information provided above. I voluntarily agree to participate in this study. After it is signed I understand that I will receive a copy of this consent form.

Signature of Research Subject

Date

Signature of Person Obtaining Consent

Date

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME Richard C. Boucher, M.D.		POSITION TITLE Professor of Medicine	
EDUCATION (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)			
INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
Yale University, New Haven, CT	B.A.	1966	Physiol. Psych.
Columbia University, New York, NY	M.D.	1970	Medicine
Columbia Presby. Hosp. New York, NY	Int/Res	1972	Medicine
Royal Victoria Hosp, Montreal, Canada	Fellow	1977	Medicine

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

Employment History:

- 1975 - 1977 Research Fellow, McGill University, Meakins-Christie Lab, Montreal, Canada
- 1977 - 1981 Assistant Professor of Medicine, University of North Carolina, Chapel Hill, NC
- 1981 - 1985 Associate Professor of Medicine, University of North Carolina, Chapel Hill, NC
- 1985 - pres. Professor of Medicine, University of North- Carolina, Chapel Hill, NC
- 1988 - pres. Director, UNC Cystic Fibrosis Center, Chapel Hill, NC
- 1990 - pres. Director, Pulmonary Division University of North Carolina, Chapel Hill, NC

Professional Societies:

- American Society for Clinical Investigation
- American Thoracic Society
- Association of American College of Physicians

Honors:

- Phi Beta Kappa
- Alpha Omega Alpha
- American Heart Established Investigator, 1980-1984
- Doris Tulcin Cystic Fibrosis Research Award, 1989

Memberships:

- 1990-pres. National Institute of Health - HLBI Research Review Committee A

Publications (23 out of 160):

1. Knowles, M.R., J.L. Carson, A.M. Collier, J.T. Gatzky and R.C. Boucher. Measurements of nasal transepithelial electric potential differences in normal human subjects *in vivo*. *Am. Rev. Respir. Dis.* 124:484-490, 1981.
2. Knowles, M.R., J.T. Gatzky and R.C. Boucher. Increased bioelectric potential difference across respiratory epithelia in cystic fibrosis. *N. Engl. J. Med.* 305:1489-1495, 1981.
3. Knowles, M., J. Gatzky and R. Boucher. Relative ion permeability of normal and cystic fibrosis nasal epithelium. *J. Clin. Invest.* 71:1410-1417, 1983.
4. Knowles, M.R., M.J. Stutts, A. Spock, N. Fischer, J.T. Gatzky and R.C. Boucher. Abnormal ion permeation through cystic fibrosis respiratory epithelium. *Science* 221:1067-1070, 1983.
5. Boucher, R.C., M.J. Stutts, M.R. Knowles, L. Cantley and J.T. Gatzky. Na⁺ transport in cystic fibrosis respiratory epithelia: Abnormal basal rate and response to adenylate cyclase activation. *J. Clin. Invest.* 78:1245-1252, 1986.

6. Iannuzzi, M.C., J.L. Weber, J. Yankaskas, R. Boucher and F.S. Collins. The introduction of biologically active foreign genes into human respiratory epithelial cells using electroporation. *Am. Rev. Respir. Dis.* 138:965-968, 1988.
7. Jetten, A.M., J.R. Yankaskas, M.J. Stutts, N.J. Willumsen and R.C. Boucher. Persistence of abnormal Cl^- conductance regulation in transformed cystic fibrosis epithelia. *Science* 244:1472-1475, 1989.
8. Carson, J.L., A.M. Collier, T.M. Gambling, M.R. Knowles and R.C. Boucher. Ultrastructure of airway epithelial cell membranes among patients with cystic fibrosis. *Human Path.* 21:640-647, 1990.
9. Knowles, M.R., N.L. Church, W.E. Waltner, J.R. Yankaskas, P. Gilligan, M. King, L.J. Edwards, R.W. Helms and R.C. Boucher. A pilot study of aerosolized amiloride for the treatment of lung disease in cystic fibrosis. *N. Engl. J. Med.* 322:1189-1194, 1990.
10. Knowles, M.R., L.L. Clarke, and R.C. Boucher. Extracellular nucleotides activate chloride secretion in cystic fibrosis airway epithelia. *N. Engl. J. Med.* 325:533-538, 1991.
11. Koller, B.H., H.S. Kim, A.M. Latour, K. Brigman, R.C. Boucher, P. Scamber, B. Wainwright, and O. Smithies. Toward an animal model of cystic fibrosis: Targeted interruption of exon 10 of cystic fibrosis transmembrane regulator gene in embryonic stem cells. *Proc. Natl. Acad. Sci. USA* 88:10730-10734, 1991.
12. Johnson, L.G., J.C. Olsen, B. Sarkadi, K.L. Moore, R. Swanstrom, and R.C. Boucher. Efficiency of gene transfer required to restore normal airway epithelial function in cystic fibrosis. *Nature Genetics* 2(1): 21-25, 1992.
13. Englehardt, J.F., J.R. Yankaskas, S.A. Ernst, Y. Yang, C.R. Marino, R.C. Boucher, J.A. Cohn, and J.M. Wilson. Submucosal glands are the predominate site of CFTR expression in human bronchus. *Nature Genetics* 2:240-247, 1992.
14. Olsen, J.C., L.G. Johnson, M.J. Stutts, B. Sarkadi, J.R. Yankaskas, R. Swanstrom, and R.C. Boucher. Correction of the apical membrane chloride permeability defect in polarized cystic fibrosis airway epithelia following retroviral-mediated gene transfer. *Human Gene Therapy* 3:253-266, 1992.
15. Sarkadi, B., D.D. Bauzon, W. Huckle, H.S. Earp, A. Berry, H. Suchindran, E. Price, J. Olsen, R.C. Boucher, and G.A. Scarborough. Biochemical characterization of the CFTR in normal and cystic fibrosis epithelial cells. *J. Biol. Chem.* 267:2087-2095 1992.
16. Curiel, D.T., S. Agarwal, N. Romer, E. Wagner, M. Cotten, M.L. Birnstiel, and R.C. Boucher. Gene transfer to respiratory epithelial cells via the receptor-mediated endocytosis pathway. *Am. J. Respir. Cell. Mol. Biol.* 6:247-252 1992.
17. Stutts, M.J., T.C. Chinet, S.J. Mason, J.M. Fullton, L.L. Clarke, and R.C. Boucher. Regulation of chloride channels in normal and cystic fibrosis airway epithelial cells by extracellular ATP. *Proc. Natl. Acad. Sci. USA* 89:1621-1625, 1992.
18. Snouwaert, J., K. Brigman, A.M. Latour, N.N. Malouf, R.C. Boucher, O. Smithies, and B.H. Koller. An animal model for CF made by gene targeting. *Science* 257:1083-1088, 1992.
19. Clarke, L.L., B. Grubb, S. Gabriel, O. Smithies, B.H. Koller, and R.C. Boucher. Defective epithelial chloride transport in a gene-targeted mouse model of cystic fibrosis. *Science* 257:1125-1128, 1992.
20. Clarke, L.L., B.R. Grubb and R.C. Boucher. An alternative non-CFTR mediated apical membrane chloride conductance in CFTR(-/-) mice. *Nature* (Submitted) 1992.
21. Gabriel, S.E., L.L. Clarke, R.C. Boucher and M.J. Stutts. CFTR and outward rectifying chloride channels are distinct proteins with a regulatory relationship. *Nature* (Submitted) 1992.
22. Olsen, J.C., L.G. Johnson, M.L. Wong-Sun, K.L. Moore, R. Swanstrom, and R.C. Boucher. Retrovirus-mediated gene transfer to cystic fibrosis airway epithelial cells: Effect of selectable marker sequences on long-term expression. *Nucleic Acids Research* (In Press) 1992.
23. Bayle, J.-Y., L.G. Johnson, J. St. George, R.C. Boucher, and J.C. Olsen. High efficiency gene transfer to primary monkey airway epithelial cells with retrovirus vectors using the GALV receptor. *Hum. Gene Therapy* (submitted, 1992.)

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME		POSITION TITLE	
Michael R. Knowles, M.D.		Associate Professor of Medicine	
EDUCATION (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)			
INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
University of North Carolina, Chapel Hill, NC	A.B.	1967	Chemistry
University of North Carolina, Chapel Hill, NC	M.D.	1971	Medicine
Ohio State University, Columbus, OH	Int. Med	1973	Medicine
Duke University, Durham, NC	Resident	1975	Medicine
University of North Carolina, Chapel Hill, NC	Fellow	1980	Pulmonary Medicine

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

Employment History:

1975-1978 Chief, Internal Medicine, U.S. Air Force, Malcolm Grow Medical Center, Andrews AFB, Washington, DC

1980-1982 Instructor, Department of Medicine, University of North Carolina-Chapel Hill

1982-1987 Assistant Professor, Medicine, Department of Medicine, UNC-Chapel Hill

1987-Present Associate Professor, Medicine, Department of Medicine, UNC-Chapel Hill

Awards and Special Appointments:

Morehead Scholar, University of North Carolina, 1963-1967

Instructor, Medicine, Uniformed Services University of Health Sciences, Bethesda, MD, 1977-78

Jefferson Pilot Fellowship in Academic Medicine, UNC-Chapel Hill, 1983-87

Research Sabbatical: Laboratory of Molecular Neurobiology, Medical Research Council and Department of Pharmacology, Cambridge University, England, 1988-89

Visiting Clinical Scholar,, Heart-Lung Transplantation Unit, Papworth Hospital, Cambridge, 1988-89

Publications: (20 of 83)

- Knowles, M.R., P. Gilligan, and R.C. Boucher. Cystic fibrosis. In: Principles and Practice of Infectious Diseases. G.L. Mandell, R.G. Douglas, Jr., and J.E. Bennett, editors. 3rd ed. Churchill Livingstone, Inc., New York. pp 579-589, 1989.
- Boucher, R.C., E.H.C. Cheng, M.J. Stutts, M.R. Knowles, and H.S. Earp. The chloride secretory response of cystic fibrosis (CF) human airway epithelia: Preservation of calcium ionophore but not protein kinase C and A-Dependent mechanisms. *J. Clin. Invest.* 84:1424-1431, 1989.
- Knowles, M.R., T.B. Barnett, A. McConkie, C. Sawyer, and S.G. Kahler. Mild cystic fibrosis in a consanguineous family: Clinical aspects. *Ann. Intern. Med.* 110:599-605, 1989.
- McConkie-Rosell, A., Y.T. Chen, M.R. Knowles, L. Silverman, D. Harris, M.C. Speer, M.A. Pericak-Vance, J.H. Ding, and S.G. Kahler. Mild cystic fibrosis linked to chromosome 7q22 markers with an uncommon haplotype. *Ann. Intern. Med.* 111:797-801, 1989.
- Orlando, R.C., D.W. Powell, R.D. Croom, H.M. Berschneider, R.C. Boucher and M.R. Knowles. Colonic and esophageal transepithelial potential difference in cystic fibrosis. *Gastroenterology* 96:1041-1048, 1989.

6. Knowles, M.R., N.L. Church, W.E. Waltner, J.R. Yankaskas, P. Gilligan, M. King, L.J. Edwards R.W. Helms, and R.C. Boucher. A pilot study of aerosolized amiloride for the treatment of lung disease in cystic fibrosis. *N. Engl. J. Med.* 322:1189-1194, 1990.
7. Kobayashi, K., M.R. Knowles, R.C. Boucher, W.E. O'Brien, and A.L. Beaudet. Benign missense mutations in the cystic fibrosis gene. *Am. J. Hum. Genet.* 47:611-615, 1990.
8. Highsmith, Jr., W.E., G.L. Chong, H.T. Orr, T.R. Perry, D. Schaid, R. Farber, K. Wagner, M.R. Knowles, W.J. Warwick, L.M. Silverman, and S.N. Thibodeau. Frequency of the Δ Phe 508 mutation and correlation with XV.2c/KM-19 haplotypes in an American population of cystic fibrosis patients: results of a collaborative study. *Clin. Chem.* 36:1741-1746, 1990.
9. Carson, J.L., A.M. Collier, T.M. Gambling, M.R. Knowles, and R.C. Boucher. Ultrastructure of airway epithelial cell membranes among patients with cystic fibrosis. *Human Path.* 21:640-647, 1990.
10. Boundy, M.G., D. Leith, and M. Knowles. Dispersion and size distribution of amiloride by metered dose and dry powder inhalers. *J. Aerosol Med.* 3:233-241, 1990.
11. Knowles, M.R., L.L. Clarke, and R.C. Boucher. Activation by extracellular nucleotides of chloride secretion in the airway epithelia of patients with cystic fibrosis. *N. Engl. J. Med.* 325:533-538, 1991.
12. Strong, T.V., L.S. Smit, S.V. Turpin, J.L. Cole, C.T. Hon, T.L. Petty, M.W. Craig, E.C. Rosenaw, III, L.-C. Tsui, M.C. Iannuzzi, M.R. Knowles, and F.S. Collins. Cystic fibrosis gene in two sisters with mild disease and normal sweat electrolyte levels. *N. Engl. J. Med.* 325: 1630-1634, 1991.
13. Gowen, C.W., Jr., M.A. Gowen, and M.R. Knowles. Colonic transepithelial potential difference in infants with cystic fibrosis. *J. Pediatr.* 118: 412-415, 1991.
14. Kilby, J.M., P.H. Gilligan, J.R. Yankaskas, W.E. Highsmith, Jr., L.J. Edwards, and M.R. Knowles. Nontuberculous mycobacteria in adult patients with cystic fibrosis. *Chest* 102:70-75, 1992.
15. Stutts, M.J., M.R. Knowles, T. Chinet, and R.C. Boucher. Abnormal ion transport in cystic fibrosis airway epithelium. In: The Airway Epithelium. Physiology, Pathophysiology, and Pharmacology. S.G. Farmer and D. W. P. Hay, editors. Marcel Dekker, Inc., New York. 301-334, 1991.
16. Knowles, M.R., L.L. Clarke, and R.C. Boucher. Extracellular ATP and UTP induce chloride secretion in nasal epithelia of CF patients and normal subjects *in vivo*. *Chest* 101:60S-63S, 1992.
17. Knowles, M.R., N.L. Church, W.E. Waltner, D. Jones, J.T. Gatzky, and R.C. Boucher. Amiloride in cystic fibrosis: safety, pharmacokinetics, and efficacy in the treatment of pulmonary disease. In: Amiloride and its Analogs: Unique Cation Transport Inhibitors. VCH Publishers, New York (In Press) 1992.
18. Turpin, S.V., and M.R. Knowles. Treatment of Pulmonary disease in patients with cystic fibrosis. In: Lung Biology in Health and Disease: Cystic Fibrosis. P.B. Davis, editor, Marcel Dekker Press Inc. (In Press) 1992.
19. Tomkiewicz, R. P., E. M. App, R. C. Boucher, M. R. Knowles, and M. King. Amiloride inhalation therapy and its influence on sputum electrolyte composition in cystic fibrosis. (Submitted) *Am. Rev. Respir. Dis.* 1992.
20. Whittier, S., R. L. Hopfer, M. R. Knowles, and P. H. Gilligan. Improved recovery of mycobacteria from respiratory secretions of patients with cystic fibrosis. (Submitted) *American Society for Microbiology*, 1992.
21. Highsmith, W.E., L.H. Burch, T.H. Boat, A. Spock, J. Gorvay, L. Quittell, K.J. Freidman, L.M. Silverman, R.C. Boucher, and M.R. Knowles. A novel cystic fibrosis gene mutation that is common in patients with normal sweat chloride concentrations. (Submitted) *N. Engl. J. Med.* 1992

Recombinant DNA Research: Actions under the Guidelines

AGENCY: National Institutes of Health, PHS, DHHS.

ACTION: Notice of actions under the NIH Guidelines for Research Involving Recombinant DNA Molecules.

SUMMARY: This notice sets forth eight actions to be taken by the Director, National Institutes of Health (NIH), under the May 7, 1986, NIH Guidelines for Research Involving Recombinant DNA Molecules (51 FR 16958).

FOR FURTHER INFORMATION CONTACT: Additional information can be obtained from Dr. Nelson A. Wivel, Director, Office of Recombinant DNA Activities (ORDA), Office of Science Policy and Technology Transfer, National Institutes of Health, Building 31, room 4B11, Bethesda, Maryland 20892, (301) 496-9838.

SUPPLEMENTARY INFORMATION: Today eight actions are being promulgated under the *NIH Guidelines for Research Involving Recombinant DNA Molecules*. These eight proposed actions were published for comment in the Federal Register of November 2, 1992 (57 FR 49584), and February 12, 1993 (58 FR 8500), and reviewed and recommended for approval by the NIH Recombinant DNA Advisory Committee (RAC) at its

meetings on December 3-4, 1992, and March 1-2, 1993.

I. Background Information and Decisions on Actions Under the NIH Guidelines

A. Addition of Appendix D-XXXX to the NIH Guidelines

In a letter dated October 5, 1992, Dr. Michael J. Welsh, Howard Hughes Medical Institution Research Laboratories, Iowa City, Iowa, submitted a human gene transfer protocol to the RAC for formal review and approval. The title of this protocol is: *Cystic Fibrosis Gene Therapy Using an Adenovirus Vector: In Vivo Safety and Efficacy in Nasal Epithelium*. This request was published for comment in the Federal Register on November 2, 1992 (57 FR 49584).

The protocol was reviewed during the RAC meeting on December 3-4, 1992. By a vote of 16 in favor, 0 opposed, and no abstentions, the RAC recommended approval of the protocol with the following stipulations: (1) Deletion of the requirement for the E1a and the rat-1 transformation assays from the protocol, (2) submission of the complete adenovirus vector sequence, and (3) incorporation of minor changes in the Informed Consent document.

On December 14, 1992, and February 15, 1993, Dr. Welsh submitted modifications and additional information to ORDA as requested by the RAC. The information was reviewed by the primary reviewers, and it was determined that the additional documentation satisfied the RAC's stipulations for approval of the protocol. The following section may be added to Appendix D:

"Appendix D-XXXX

"Dr. Michael J. Welsh, Howard Hughes Medical Institute Research Laboratories, University of Iowa College of Medicine, Iowa City, Iowa, may conduct experiments on 3 cystic fibrosis (CF) patients $\geq$ 18 years of age with mild to moderate disease. This Phase I study will determine the: (1) *In vivo* safety and efficacy of the administration of the replication-deficient type 2 adenovirus vector, Ad2/CFTR-1, to the nasal epithelium; (2) efficacy in correcting the CF chloride transport defect *in vivo*; and (3) effect of adenovirus vector dosage on safety and efficacy."

I accept this recommendation, and Appendix D-XXXX of the *NIH Guidelines* will be added accordingly.

B. Addition of Appendix D-XXXXI to the NIH Guidelines

In a letter dated October 7, 1992, Dr. Ronald G. Crystal, National Institutes of

Health, Bethesda, Maryland, submitted a human gene therapy protocol to the RAC for formal review and approval. The original title of this protocol was: *Gene Therapy of the Respiratory Manifestations of Cystic Fibrosis using a Replication Deficient, Recombinant Adenovirus to Transfer the Normal Human Cystic Fibrosis Transmembrane Conductance Regulator cDNA to the Airway Epithelium*. This request was published for comment in the Federal Register on November 2, 1992 (57 FR 49584).

The protocol was reviewed during the RAC meeting on December 3-4, 1992. By a vote of 17 in favor, 0 opposed, and no abstentions, the RAC recommended approval of the protocol with the following modifications: (1) The patient eligibility criterion requiring that patients are documented to be sterile will be replaced with a statement suggesting that all patients should exercise appropriate birth control methods, (2) include the statement, "There may be no long term benefit to patients from this procedure" in the Informed Consent Document, and (3) revise the title of the protocol to read as follows: *A Phase I Study, in Cystic Fibrosis (CF) Patients, of the Safety, Toxicity, and Biological Efficacy of a Single Administration of a Replication Deficient, Recombinant Adenovirus Carrying the cDNA of the Normal Human Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) Gene in the Lung*, and (4) demonstrate that there is less than one replication-competent adenovirus particle per 20 milliliters of supernatant (standard dosage).

On February 4, 1993, Dr. Crystal submitted the modified sections of the protocol to ORDA except for the data requested in modification number (4). Dr. Crystal stated that a lot release has been established for each preparation. Lot release forms with the relevant data will be forwarded to ORDA and FDA simultaneously. Approval from these agencies must be obtained before the clinical experiment can proceed. This information was reviewed by the RAC Executive Secretary, and it was determined that it meets the request of the RAC. The following section may be added to Appendix D:

"Appendix D-XXXXI

"Dr. Ronald G. Crystal, National Institutes of Health, Bethesda, Maryland, may conduct experiments on 10 cystic fibrosis (CF) patients $\geq$ 21 years of age. Patients will receive an initial administration of the replication-deficient type 5 adenovirus vector, AdCFTR, to their left nares. If no toxicity is observed from intranasal

administration, patients will receive a single administration of AdCFTR to the respiratory epithelium of their left large bronchi. Five groups of patients (2 patients per group) will be studied based on increased dosage administration of AdCFTR. This study will determine that: (1) *in vivo* safety and efficacy of the administration of AdCFTR into the respiratory epithelium; (2) efficacy of the correction of the biologic abnormalities of CF in the respiratory epithelium; (3) duration of the biologic correction; (4) efficacy of the correction of the abnormal electrical potential difference of the airway epithelial sheet; (5) clinical parameters relevant to the disease process; and (6) if humoral immunity develops against AdCFTR sufficient to prevent repeat administration."

I accept this recommendation, and Appendix XXXXI of the *NIH Guidelines* will be added accordingly.

C. Addition of Appendix D-XXXXII of the *NIH Guidelines*

In a letter dated December 7, 1992, Dr. Kenneth Culvar, Iowa Methodist Medical Center, Des Moines, Iowa, and Dr. John C. Van Gilder, University of Iowa, Iowa City, Iowa, indicated the intention to submit a human gene therapy protocol to the RAC for formal review and approval. The title of this protocol is: *Gene Therapy for the Treatment of Malignant Brain Tumors with In Vivo Tumor Transduction with the Herpes Simplex Thymidine Kinase Gene/Ganciclovir System*. This request was published for comment in the Federal Register on February 12, 1993 (58 FR 8500).

The protocol was reviewed during the RAC meeting on March 1-2, 1993. By a vote of 19 in favor, 0 opposed, and no abstentions, the RAC recommended approval of the protocol with the following modifications: (1) Patient eligibility will be limited to those patients who have measurable residual tumor immediately following the post-operative procedure as demonstrated by imaging studies, i.e., MRI or CT scans. (2) Patient enrollment in the protocol will be limited to 15 patients. If a positive response is observed in any of the first 15 patients, the investigators may submit a request to treat an additional 15 patients. The total number of patients treated will be divided between Iowa Methodist Medical Center and the University of Iowa. (3) Following 3 injections of herpes simplex thymidine kinase (HS-tk) vector-producing cells (VPC), patients will be eligible for additional treatments only if they have demonstrated stable disease for a minimum of 6 months. The

following section may be added to Appendix D:

"Appendix D-XXXXII

"Dr. Kenneth Culvar, Iowa Methodist Medical Center, Des Moines, Iowa, and Dr. John Van Gilder, University of Iowa, Iowa City, Iowa, may conduct experiments on 15 patients $\geq$ 18 years of age with recurrent malignant primary brain tumors or lung, melanoma, renal cell carcinoma, or breast carcinoma, brain metastases who have failed standard therapy for their disease. Patient eligibility will be limited to those patients who have measurable residual tumor immediately following the post-operative procedure as demonstrated by imaging studies. The number of patients treated will be equally divided between the Iowa Methodist Medical Center and the University of Iowa. If a positive response is observed in any of the first 15 patients, the investigators may submit a request to treat an additional 15 patients.

"Following surgical debulking, patients will receive a maximum of 3 intrathecal injections of the G1TkSvNa vector-producing cell line (VPC) to induce regression of residual tumor cells by ganciclovir (GCV) therapy. Patients who demonstrate stable disease for a minimum of 6 months following this treatment will be eligible for additional VPC injections and subsequent GCV therapy."

I accept this recommendation, and Appendix D-XXXXII of the *NIH Guidelines* will be added accordingly.

D. Addition of Appendix D-XXXXIII of the *NIH Guidelines*

In a letter dated December 31, 1992, Drs. Malcolm Brenner, Robert Krance, Halan E. Heslop, Victor Santana, and James Ihla of the St. Jude Children's Research Hospital, Memphis, Tennessee, submitted a human gene transfer protocol to the RAC for formal review and approval. The title of this protocol is: *Assessment of the Efficacy of Purging by Using Gene-Marked Autologous Marrow Transplantation for Children with Acute Myelogenous Leukemia in First Complete Remission*. This request was published for comment in the Federal Register on February 12, 1993 (58 FR 8500).

The protocol was reviewed during the RAC meeting on March 1-2, 1993. By a vote of 17 in favor, 0 opposed, and no abstentions, the RAC recommended approval of the protocol. The following section may be added to Appendix D:

"Appendix D-XXXXIII

"Drs. Malcolm Brenner, Robert Krance, Helen E. Heslop, Victor Santana, and James Ihle, St. Jude Children's Research Hospital, Memphis, Tennessee, may conduct experiments on 35 patients ≥ 1 year and ≤ 21 years of age at the time of initial diagnosis of acute myelogenous leukemia (AML). The investigators will use the two retroviral vectors, LNL6 and G1Na, to determine the efficacy of the bone marrow purging techniques: 4-hydroxyperoxycyclophosphamide and Interleukin-2 (IL-2) activation of endogenous cytotoxic effector cells, in preventing relapse from the reinfusion of autologous bone marrow cells."

I accept this recommendation, and Appendix D-XXXXIII of the NIH Guidelines will be added accordingly.

E. Addition of Appendix D-XXXXIV of the NIH Guidelines

In a letter dated December 31, 1992, Drs. Helen E. Heslop, Malcolm Brenner, and Cliona Rooney of the St. Jude Children's Research Hospital, Memphis, Tennessee, submitted a human gene transfer protocol to the RAC for formal review and approval. The title of this protocol is: *Administration of Neomycin Resistance Gene Marked EBV Specific Cytotoxic T Lymphocytes to Recipients of Mismatched-Related or Phenotypically Similar Unrelated Donor Marrow Grafts*. This request was unpublished for comment in the Federal Register on February 12, 1993 (58 FR 8500).

The protocol was reviewed during the RAC meeting on March 1-2, 1993. By a vote of 19 in favor, 0 opposed, and no abstentions, the RAC recommended approval of the protocol. The following section may be added to Appendix D.

"Appendix D-XXXXIV

"Drs. Helen E. Heslop, Malcolm Brenner, and Cliona Rooney, St. Jude Children's Research Hospital, Memphis, Tennessee, may conduct experiments on 35 patients ≤ 21 years of age who will be recipients of mismatched-related or phenotypically similar unrelated donor marrow grafts for leukemia. In this Phase I dose escalation study, spontaneous lymphoblastoid cell lines will be established that express the same range of Epstein-Barr Virus (EBV) encoded proteins as the recipient. These EBV-specific cell lines will be transduced with the LNL6 or G1Na retroviral vector and readministered at the time of bone marrow transplant. This study will determine: (1) survival and expansion of these EBV-specific cell lines *in vivo*, (2) the ability of these

adoptively transferred cells to confer protection against EBV infection, and (3) appropriate dosage and administration schedules."

I accept this recommendation, and Appendix D-XXXXIV of the NIH Guidelines will be added accordingly.

F. Addition to Appendix D-XXXXV to the NIH Guidelines

In a letter dated December 23, 1992, Drs. Robert W. Wilmott and Jeffrey Whitsett, Children's Hospital Medical Center, Cincinnati, Ohio, and Dr. Bruce Trapnell, Genetic Therapy, Inc., in Gaithersburg, Maryland, indicated the intention to submit a human gene therapy protocol to the RAC for formal review and approval. The title of this protocol is: *A Phase I study of Gene Therapy of Cystic Fibrosis Utilizing a Replication Deficient Recombinant Adenovirus Vector to Deliver the Human Cystic Fibrosis Transmembrane Conductance Regulatory cDNA to the Airways*. This request was published for comment in the Federal Register on February 12, 1993 (58 FR 8500).

The protocol was reviewed during the RAC meeting on March 1-2, 1993. By a vote of 16 in favor, 0 opposed, and 2 abstentions, the RAC recommended approval of the protocol with the following modification: (1) the second administration of the adenovirus vector (Ad1CF2), and associated procedures, will be eliminated from the protocol. The RAC recommended that the investigators should attempt to obtain a level of sensitivity adequate to detect one replication-competent virus particle per patient dose, i.e., 20 milliliter of retroviral vector supernatant.

On March 18, 1993, Dr. Wilmott submitted the modified protocol to ORDA. The modified protocol was reviewed by the RAC Executive Secretary, and it was determined that it meets the request of the RAC. The following section may be added to Appendix D:

"Appendix D-XXXXV

"Drs. Robert W. Wilmott and Jeffrey Whitsett, Children's Hospital Medical Center, Cincinnati, Ohio, and Dr. Bruce Trapnell, Genetic Therapy, Inc., Gaithersburg, Maryland, may conduct experiments on 15 cystic fibrosis (CF) patients who have mild to moderate disease ≥ 21 years of age. The replication-deficient type 5 adenovirus vector, Av1CF2, will be administered to the nasal and lobar bronchial respiratory tract of patients. This study will demonstrate the: (1) expression of normal cystic fibrosis transmembrane conductance regulator (CFTR) mRNA *in vivo*, (2) synthesis of CFTR protein, and

(3) correction of apical cell cAMP dependent Cl⁻ permeability. The pharmacokinetics of CFTR expression and ability to re-infect the respiratory tract with Av1CF2 will be determined. Systemic and local immunologic consequences of Av1CF2 infection, the time of viral survival, and potential for recombination or complementation of the virus will be monitored."

I accept this recommendation, and Appendix XXXV of the NIH Guidelines will be added accordingly.

G. Amendment to the "Points To Consider in the Design and Submission of Protocols for the Transfer of Recombinant DNA Into the Genome of Human Subjects" Regarding the Use of Compassionate Plea

In a letter dated December 7, 1992, Dr. Ivor Royston of the San Diego Regional Cancer Center, San Diego, California, requested a compassionate plea approval for a human gene therapy protocol. This RAC established a working group to develop policy regarding compassionate plea exemptions.

The Points to Consider (March 1, 1990, 55 FR 7443) provide guidance to scientists and clinical investigators submitting human gene therapy/transfer protocols. During the RAC meeting on January 14, 1993, the committee adopted the following preliminary policy statement regarding the approval of human gene therapy protocols on an expedited basis. The following original statement included the following elements which are not listed in order of importance, but are simply meant to be inclusive of the issues that need to be addressed:

"1. NIH will strongly emphasize that the standard method of protocol submission is highly preferred.

"2. The RAC will consider single patient protocols.

"3. There will be no attempt to distinguish between research and treatment in the consideration of protocols.

"4. Regardless of the method of review, the criteria must be the same for all protocols.

"5. When time-sensitive circumstances prevail, the NIH will do an internal review.

"6. To the extent that it is legally and practically possible, the Director of NIH will ask NIH experts, RAC members, and other experts to participate in protocol review.

"7. Among other factors to be considered by the Director of NIH, is the consistency of the new protocol to existing protocols.

"8. The NIH will report to the RAC following its internal review.

"9. Protocols that are deferred or not approved by the RAC in its normal review process, are not eligible for expedited review.

"10. In the development of any documents that are a part of this policy statement, the terms, compassionate use and compassionate treatment, will be deliberately avoided."

This preliminary policy statement was published for comment in the Federal Register on February 12, 1993 (58 FR 8500).

During the March 1-2, 1993, meeting, the RAC reviewed the preliminary policy statement. By a vote of 16 in favor, 0 opposed, and no abstentions, the RAC recommended that the following section be added to the Points to Consider:

"VI. Procedures to be Followed for Expedited Review

"1. An investigator submitting a request to the NIH for expedited review of a gene transfer protocol must provide detailed information regarding the necessity of expedited review.

"2. No protocol shall be considered without Institutional Biosafety Committee (IBC) and Institutional Review Board (IRB) approval.

"3. At this time, all gene transfer protocols must be considered experimental.

"4. Regardless of the method of review, the *Points to Consider* must be the standard of review for all gene transfer protocols.

"5. Review of such protocols may include intramural NIH experts but must include extramural experts.

"6. Among other factor to be considered by the reviewers, is the similarity of the new protocol to previously approved protocols.

"7. The NIH will report to the RAC following expedited review and will include all of the materials on which the decision was based. The RAC will formally review the protocol at its next scheduled meeting. Patient privacy will be maintained.

"8. Protocols that are deferred or not approved by the RAC in its normal review process are not eligible for expedited review. No protocol shall have more than one patient approved under expedited review.

"9. As requested in the context of non-expedited review, none of the costs of the experimental protocol should be borne by the patient or the patient's family."

"10. Data on all patients undergoing gene transfer shall be provided to the RAC within six months of the procedure."

I accept this recommendation, and the *Points to Consider of the NIH Guidelines* will now contain this new addition.

H. Amendment to the Points To Consider Regarding the Separation of the Gene Marking Informed Consent Document From the Therapeutic Informed Consent Documents

During the September 14-15, 1992, RAC meeting, Dr. Leonard Post requested that when a gene transfer protocol is submitted as an addition to an IRB-approval clinical protocol, the principal investigator should submit two separate informed consent documents, one for the gene marking procedures and one for the therapeutic portion of the protocol. In the *Points to Consider, Part I-D—Informed Consent* (March 1, 1990, 55 FR 7446), a new sentence would be added to the introductory paragraph:

"When gene transfer is a procedure separate from the therapeutic protocol, an informed consent document should be submitted for both the gene marking and therapeutic procedures."

This request was published for comment in the Federal Register on February 12, 1993 (58 FR 8500).

The request was reviewed during the RAC meeting on March 1-2, 1993. By a vote of 17 in favor, 0 opposed, and no abstentions, the RAC recommended that the following sentence be added to the introductory paragraph of Section I-D—Informed Consent:

"When gene transfer is a procedure separate from a clinical protocol, informed consent documents should be submitted for both the gene transfer and clinical protocols."

I accept this recommendation and the *Points to Consider of the NIH Guidelines* will now contain this new addition.

II. Summary of Actions

A. Addition of Appendix D-XXXX to the NIH Guidelines

The following section is added to Appendix D:

"Dr. Michael J. Welsh, Howard Hughes Medical Institute Research Laboratories, University of Iowa College of Medicine, Iowa City, Iowa, may conduct experiments on 3 cystic fibrosis (CF) patients $\geq$ 18 years of age with mild to moderate disease. This Phase I study will determine the: (1) *in vivo* safety and efficacy of the administration of the replication-deficient type 2 adenovirus vector, Ad2/CFTR-1, to the nasal epithelium; (2) efficacy in correcting the CF chloride transport defect *in vivo*; and (3) effect of adenovirus vector dosage on safety and efficacy."

B. Addition of Appendix D-XXXXI to the NIH Guidelines

The following section is added to Appendix D:

"Dr. Ronald G. Crystal, National Institutes of Health, Bethesda, Maryland, may conduct experiments on 10 cystic fibrosis (CF) patients $\geq$ 21 years of age. Patients will receive an initial administration of the replication-deficient type 5 adenovirus vector, AdCFTR, to their left nares. If no toxicity is observed from intranasal administration, patients will receive a single administration of AdCFTR to the respiratory epithelium of their left large bronchi. Five groups of patients (2 patients per group) will be studied based on increased dosage administration of AdCFTR. This study will determine the: (1) *in vivo* safety and efficacy of the administration of AdCFTR into the respiratory epithelium; (2) efficacy of the correction of the biologic abnormalities of CF in the respiratory epithelium; (3) duration of the biologic correction; (4) efficacy of the correction of the abnormal electrical potential difference of the airway epithelial sheet; (5) clinical parameters relevant to the disease process; and (6) if humoral immunity develops against AdCFTR sufficient to prevent repeat administration."

C. Addition of Appendix D-XXXXII of the NIH Guidelines

The following section is added to Appendix D:

"Dr. Kenneth Culver, Iowa Methodist Medical Center, Des Moines, Iowa, and Dr. John Van Gilder, University of Iowa, Iowa City, Iowa, may conduct experiments on 15 patients $\geq$ 18 years of age with recurrent malignant primary brain tumors or lung, melanoma, renal cell carcinoma, or breast carcinoma brain metastases who have failed standard therapy for their disease. Patient eligibility will be limited to those patients who have measurable residual tumor immediately following the post-operative procedure as demonstrated by imaging studies. The number of patients treated will be equally divided between the Iowa Methodist Medical Center and the University of Iowa. If a positive response is observed in any of the first 15 patients, the investigators may submit a request to treat an additional 15 patients.

"Following surgical debulking, patients will receive a maximum of 3 intralesional injections of the G1TkSvNa vector-producing cell line (VPC) to induce regression of residual tumor cells by ganciclovir (GCV) therapy."

Patients who demonstrate stable disease for a minimum of 6 months following this treatment will be eligible for additional VPC injections and subsequent GCV therapy."

D. Addition of Appendix D-XXXXIII of the NIH Guidelines

The following section is added to Appendix D:

"Drs. Malcolm Brenner, Robert Krance, Helen E. Heslop, Victor Santana, and James Ihle, St. Jude Children's Research Hospital, Memphis, Tennessee, may conduct experiments on 35 patients ≥ 1 year and ≤ 21 years of age at the time of initial diagnosis of acute myelogenous leukemia (AML). The investigators will use the two retroviral vectors, LNL6 and G1Na, to determine the efficacy of the bone marrow purging techniques: 4-hydroxypyroxycyclophosphamide and interleukin-2 (IL-2) activation of endogenous cytotoxic effector cells, in preventing relapse from the reinfusion of autologous bone marrow cells."

E. Addition of Appendix D-XXXXIV of the NIH Guidelines

The following section is added to Appendix D:

"Drs. Helen E. Heslop, Malcolm Brenner, and Cliona Rooney, St. Jude Children's Research Hospital, Memphis, Tennessee, may conduct experiments of 35 patients ≤ 21 years of age who will be recipients of mismatched-related or phenotypically similar unrelated donor marrow grafts for leukemia. In this Phase I dose escalation study, spontaneous lymphoblastoid cell lines will be established that express the same range of Epstein-Barr Virus (EBV) encoded proteins as the recipient. These EBV-specific cell lines will be transduced with LNL6 or G1Na and readministered at the time of bone marrow transplant. This study will determine: (1) survival and expansion of these EBV-specific cell lines *in vivo*, (2) the ability of these adoptively transferred cells to confer protection against EBV infection, and (3) appropriate dosage and administration schedules."

F. Addition to Appendix D-XXXXV of the NIH Guidelines

The following section is added to Appendix D:

"Drs. Robert W. Wilmott and Jeffrey Whitsett, Children's Hospital Medical Center, Cincinnati, Ohio, and Dr. Bruce Trapnell, Genetic Therapy, Inc., Gaithersburg, Maryland, may conduct experiments on 15 cystic fibrosis (CF) patients who have mild to moderate disease ≥ 21 years of age. The

replication-deficient type 5 adenovirus vector, Av1CF2, will be administered to the nasal and lobar bronchial respiratory tract of patients. This study will demonstrate the: (1) expression of normal cystic fibrosis transmembrane conductance regulator (CFTR) mRNA *in vivo*, (2) synthesis of CFTR protein, and (3) correction of epithelial cell cAMP dependent Cl permeability. The pharmacokinetics of CFTR expression and ability to re-infect the respiratory tract with AvCF2 will be determined. Systematic and local immunologic consequences of Av1CF2 infection, the time of viral survival, and potential for recombination or complementation of the virus will be monitored."

G. Amendment to the "Points To Consider in the Design and Submission of Protocols for the Transfer of Recombinant DNA Into the Genome of Human Subjects" Regarding the Use of Compassionate Plea

The following section is added to the Points to Consider of the NIH Guidelines:

"VI. Procedures to be Followed for Expedited Review

"1. An investigator submitting a request to the NIH for expedited review of a gene transfer protocol must provide detailed information regarding the necessity of expedited review.

"2. No protocol shall be considered without Institutional Biosafety Committee (IBC) and Institutional Review Board (IRB) approval.

"3. At this time, all gene transfer protocols must be considered experimental.

"4. Regardless of the method of review, the Points to Consider must be the standard of review for all gene transfer protocols.

"5. Review of such protocols may include intramural NIH experts but must include extramural experts.

"6. Among other factors to be considered by the reviewers, is the similarity of the new protocol to previously approved protocols.

"7. The NIH will report to the RAC following expedited review and will include all of the materials on which the decision was based. The RAC will formally review the protocol at its next scheduled meeting. Patient privacy will be maintained.

"8. Protocols that are deferred or not approved by the RAC in its normal review process are not eligible for expedited review. No protocol shall have more than one patient approved under expedited review.

"9. As requested in the context of non-expedited review, none of the costs

of the experimental protocol should be borne by the patient or the patient's family.

"10. Data on all patients undergoing gene transfer shall be provided to the RAC within six months of the procedure."

H. Amendment to the Points To Consider Regarding the Separation of the Gene Marking Informed Consent Document From the Therapeutic Informed Consent Documents

In the Points to Consider, Part I-D—Informed Consent (March 1, 1990, 55 FR 7446), a new sentence would be added to the introductory paragraph:

"When gene transfer is a procedure separate from a clinical protocol, informed consent documents should be submitted for both the gene transfer and clinical protocols."

OMB's "Mandatory Information Requirements for Federal Assistance Program Announcements" (45 FR 39592) requires a statement concerning the official government programs contained in the Catalog of Federal Domestic Assistance. Normally NIH lists in its announcements the number and title of affected individual programs for the guidance of the public. Because the guidance in this notice covers not only virtually every NIH program but also essentially every Federal research program in which DNA recombinant molecule techniques could be used, it has been determined to be not cost effective or in the public interest to attempt to list these programs. Such a list would likely require several additional pages. In addition, NIH could not be certain that every Federal program would be included as many Federal agencies, as well as private organizations, both national and international, have elected to follow the NIH Guidelines. In lieu of the individual program listing, NIH invites readers to direct questions to the information address above about whether individual programs listed in the Catalog of Federal Domestic Assistance are affected.

Effective Date: April 16, 1993.

Bernadine Healy,

Director, National Institutes of Health.

[FR Doc. 93-9503 Filed 4-22-93; 8:45 am]

BILLING CODE 4140-01-M

Testis Report

Tuesday
May 4, 1993

Part V

Department of Health and Human Services

National Institutes of Health

Recombinant DNA Advisory Committee
Meeting; Recombinant DNA Research;
Proposed Actions Under the Guidelines;
Notices

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

Recombinant DNA Advisory Committee; Meeting

Pursuant to Public Law 92-463, notice is hereby given of a meeting of the Recombinant DNA Advisory Committee on June 7-8, 1993. The meeting will be held at the National Institutes of Health, Building 31C, Conference Room 6, 9000 Rockville Pike, Bethesda, Maryland 20892, starting at approximately 9 a.m. on June 7, 1993, to adjournment at approximately 5 p.m. on June 8, 1993. The meeting will be open to the public to discuss the following proposed actions under the *NIH Guidelines for Research Involving Recombinant DNA Molecules* (51 FR 16958):

Proposed Major Actions to the NIH Guidelines

Additions to Appendix D of the *NIH Guidelines* regarding thirteen (13) Human Gene Therapy/Gene Transfer Protocols;

Additions to Appendix D of the *NIH Guidelines* Regarding Semliki Forest Virus; Poxvirus Vectors-NYVAC, ALVAC, TROVAC, and Reicketsia prowazeki;

Amendment to Section IV-C-3-c of the *NIH Guidelines* Regarding the *Recombinant DNA Technical Bulletin* and the Data Management of the NIH-Approved Human Gene Transfer Protocols;

Amendments to the *Points to Consider in the Design and Submission of Protocols for the Transfer of Recombinant DNA into the Genome of Human Subjects* Regarding Reporting Requirements for Human Gene Transfer/Gene Therapy Protocols; the term "Subject(s)"; Satellite Institutions; Categorization of Protocols; and Protocol Format.

Other Matters To Be Considered by the Committee

Attendance by the public will be limited to space available. Members of the public wishing to speak at this meeting may be given such opportunity at the discretion of the Chair.

Dr. Nelson A. Wivel, Director, Office of Recombinant DNA Activities, National Institutes of Health, Building 31, room 4B11, Bethesda, Maryland 20892, Phone (301) 496-9838, FAX (301) 496-9839, will provide materials to be discussed at this meeting, roster of committee members, and substantive program information. Individuals who plan to attend and need special

assistance, such as sign language interpretation or other reasonable accommodations, should contact Dr. Wivel in advance of the meeting. A summary of the meeting will be available at a later date.

OMB's "Mandatory Information Requirements for Federal Assistance Program Announcements" (45 FR 39592, June 11, 1980) requires a statement concerning the official government programs contained in the *Catalog of Federal Domestic Assistance*. Normally NIH lists in its announcements the number and title of affected individual programs for the guidance of the public. Because the guidance in this notice covers not only virtually every NIH program but also essentially every Federal research program in which DNA recombinant molecule techniques could be used, it has been determined not to be cost effective or in the public interest to attempt to list these programs. Such a list would likely require several additional pages. In addition, NIH could not be certain that every Federal program would be included as many Federal agencies, as well as private organizations, both national and international, have elected to follow the *NIH Guidelines*. In lieu of the individual program listing, NIH invites readers to direct questions to the information address above about whether individual programs listed in the *Catalog of Federal Domestic Assistance* are affected.

Dated: April 28, 1993.

Susan K. Feldman,
Committee Management Officer, NIH.
[FR Doc. 93-10487 Filed 5-3-93; 8:45 am]
BILLING CODE 4140-01-M

Recombinant DNA Research: Proposed Actions Under the Guidelines

AGENCY: National Institutes of Health, PHS, DHHS.

ACTION: Notice of proposed actions under the NIH Guidelines for Research Involving Recombinant DNA Molecules (51 FR 16958).

SUMMARY: This notice sets forth proposed actions to be taken under the National Institutes of Health (NIH) Guidelines for Research Involving Recombinant DNA Molecules (51 FR 16958). Interested parties are invited to submit comments concerning these proposals. These proposals will be considered by the Recombinant DNA Advisory Committee (RAC) at its meeting on June 7-8, 1993. After consideration of these proposals and comments by the RAC, the Director of

the National Institutes of Health will issue decisions in accordance with the NIH Guidelines.

DATES: Comments received by May 25, 1993, will be reproduced and distributed to the RAC for consideration at its June 7-8, 1993, meeting.

ADDRESSES: Written comments and recommendations should be submitted to Dr. Nelson A. Wivel, Director, Office of Recombinant DNA Activities (ORDA), Building 31, room 4B11, National Institutes of Health, Bethesda, Maryland 20892, or sent by FAX to 301-496-9839.

All comments received in timely response to this notice will be considered and will be available for public inspection in the above office on weekdays between the hours of 8:30 a.m. and 5 p.m.

FOR FURTHER INFORMATION CONTACT: Background documentation and additional information can be obtained from the Office of Recombinant DNA Activities, Building 31, room 4B11, National Institutes of Health, Bethesda, Maryland 20892, (301) 496-9838.

SUPPLEMENTARY INFORMATION: The NIH will consider the following actions under the NIH Guidelines for Research Involving Recombinant DNA Molecules:

I. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Therapy Protocol/Drs. Deisseroth, Kavanagh, Champlin

In a letter dated March 4, 1993, Drs. Albert B. Deisseroth, John Kavanagh, and Richard Champlin of the University of Texas MD Anderson Cancer Center, Houston, Texas, indicated their intention to resubmit a human gene therapy protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: Use of Safety-Modified Retroviruses to Introduce Chemotherapy Resistance Sequences into Normal Hematopoietic Stem Cells for Chemoprotection During the Therapy of Ovarian Cancer: A Pilot Trial.

II. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Therapy Protocol/Drs. Das Gupta, Cohen, Richards

In a letter dated February 26, 1993, Drs. Tapas K. Das Gupta and Edward P. Cohen of the University of Illinois College of Medicine, Chicago, Illinois, and Dr. Jon M. Richards of the University of Chicago, Chicago, Illinois, submitted a human gene therapy protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: Immunization of Malignant Melanoma Patients with Interleukin-2-

Secreting Melanoma Cells Expressing Defined Allogeneic Histocompatibility Antigens.

III. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Therapy Protocol/Drs. Galpin, Casciato

On April 9, 1993, a human gene therapy protocol was submitted for Drs. Jeffrey E. Galpin of the University of Southern California, Los Angeles, California, and Dr. Dennis A. Casciato of the University of California, Los Angeles, California, by Dr. Bruce Merchant, Viagene, Inc., San Diego, California (sponsor of the protocol), to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: *A Preliminary Study to Evaluate the Safety and Biologic Effects of Murine Retroviral Vector Encoding HIV-1 Genes [HIV-IT(V)] in Asymptomatic Subjects Infected with HIV-1.*

IV. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Therapy Protocol/Drs. Karlsson, Dunbar, Kohn

In a letter dated April 10, 1993, Drs. Stefan Karlsson and Cynthia Dunbar of the National Institutes of Health, Bethesda, Maryland, and Dr. Donald B. Kohn of Childrens Hospital Los Angeles, Los Angeles, California, submitted a human gene therapy protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: *Retroviral Mediated Transfer of the cDNA for Human Glucocerebrosidase into Hematopoietic Stem Cells of Patients with Gaucher Disease.*

V. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Therapy Protocol/Dr. Barranger

In a letter dated March 26, 1993, Dr. John A. Barranger of the University of Pittsburgh, Pittsburgh, Pennsylvania, indicated his intention to submit a human gene therapy protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: *Gene Therapy for Gaucher Disease: Ex Vivo Gene Transfer and Autologous Transplantation of CD34(+) Cells.*

VI. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Therapy Protocol/Drs. Raffel, Culver

In a letter dated April 5, 1993, Dr. Corey Raffel of the Childrens Hospital Los Angeles, Los Angeles, California, and Dr. Kenneth Culver of Iowa Methodist Medical Center, Des Moines, Iowa, submitted a human gene therapy protocol to the Recombinant DNA

Advisory Committee for formal review and approval. The title of this protocol is: *Gene Therapy for the Treatment of Recurrent Pediatric Malignant Astrocytomas with In Vivo Tumor Transduction with the Herpes Simplex Thymidine Kinase Gene.*

VII. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Therapy Protocol/Drs. Hesdorffer, Antman

In a letter dated March 8, 1993, Dr. Arthur Bank of Columbia University, New York, New York, indicated the intention of Drs. Charles Hesdorffer and Karen Antman of the Columbia University, College of Physicians and Surgeons, New York, New York, to submit a human gene therapy protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: *Human MDR Gene Transfer in Patients with Advanced Cancer.*

VIII. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Transfer Protocol/Dr. Freedman

In a letter dated March 22, 1993, Dr. Ralph S. Freedman of the University of Texas MD Anderson Cancer Center, Houston, Texas, indicated the intention to submit a human gene transfer protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: *Use of a Retroviral Vector to Study the Trafficking Patterns of Purified Ovarian TIL Populations Used in Intraperitoneal Adoptive Immunotherapy of Ovarian Cancer Patients—A Pilot Study.*

IX. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Therapy Protocol/Dr. Ilan

In a letter dated April 10, 1993, Dr. Joseph Ilan of the Case Western Reserve University School of Medicine and University Hospitals of Cleveland, Cleveland, Ohio, submitted a human gene therapy protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: *Gene Therapy for Human Brain Tumors Using Episome-Based Antisense cDNA Transcription of Insulin-Like Growth Factor I.*

X. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Therapy Protocol/Dr. Nabel

In a letter dated April 9, 1993, Dr. Gary J. Nabel of the University of Michigan Medical Center, Ann Arbor, Michigan, submitted a human gene therapy protocol to the Recombinant

DNA Advisory Committee for formal review and approval. The title of this protocol is: *Immunotherapy for Cancer by Direct Gene Transfer into Tumors.*

XI. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Therapy Protocol/Dr. Nabel

In a letter dated April 9, 1993, Dr. Gary J. Nabel of the University of Michigan Medical Center, Ann Arbor, Michigan, submitted a human gene therapy protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: *A Molecular Genetic Intervention for AIDS—Effects of a Transdominant Negative Form of Rev.*

XII. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Therapy Protocol/Dr. Seigler

In a letter dated April 9, 1993, Dr. Hilliard F. Seigler of the Duke University Medical Center, Durham, North Carolina, resubmitted a human gene therapy protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: *A Phase I Trial of Human Gamma Interferon-Transduced Autologous Tumor Cells in Patients With Disseminated Malignant Melanoma.*

XIII. Amendment to Appendix D—XV of the NIH Guidelines Regarding a Human Gene Therapy Protocol/Dr. Blaese

On April 8, 1993, an amendment to the human gene therapy protocol was submitted for Dr. R. Michael Blaese of the National Institutes of Health, Bethesda, Maryland, by Dr. Craig A. Mullen of the National Institutes of Health, Bethesda, Maryland, to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: *Treatment of Severe Combined Immune Deficiency (SCID) due to Adenosine Deaminase (ADA) Deficiency with CD34(+) Selected Autologous Hematopoietic Stem Cells.*

XIV. Addition to Appendix D of the NIH Guidelines Regarding Semliki Forest Virus/Dr. Temple

In a letter dated February 8, 1993, Dr. Gary F. Temple of Life Technologies, Inc., Gaithersburg, Maryland, submitted a request for a reduction in physical containment from Biosafety Level 3 to Biosafety Level 2 for a Semliki Forest Virus vector expression system.

XV. Addition to Appendix D of the NIH Guidelines Regarding Poxvirus Vectors—NYVAC, ALVAC, TROVAC/Dr. Trag

In a letter dated January 8, 1993, Dr. Arvilla L. Trag of Virogenetics Corporation, Troy, New York, submitted a request for reduction in physical containment from Biosafety Level 2 to Biosafety Level 1 for three poxvirus vectors: NYVAC, ALVAC, TROVAC.

XVI. Addition to Appendix D of the NIH Guidelines Regarding Rickettsia prowazeki/Dr. Policastro

In a letter dated March 23, 1993, Dr. Paul Policastro of the National Institutes of Health, Bethesda, Maryland, submitted a request to transfer a chloramphenicol resistance marker to an avirulent strain of Rickettsia prowazeki.

XVII. Amendment to Section IV-C-3-c of the NIH Guidelines Regarding the Recombinant DNA Technical Bulletin and the Data Management of the NIH-Approved Human Gene Transfer Protocols/Ms. Wilson

In a letter dated April 5, 1993, Debra Wilson of the National Institutes of Health submitted a request that Section IV-C-3-c be amended to delete the requirement that the Office of Recombinant DNA Activities (ORDA) publish the *Recombinant DNA Technical Bulletin*, and to add the requirement that ORDA will serve as the focal point for data management of the NIH-approved human gene transfer protocols.

XVIII. Amendment to the Points to Consider in the Design and Submission of Protocols for the Transfer of Recombinant DNA Into the Genome of Human Subjects Regarding Reporting Requirements for Human Gene Transfer/Gene Therapy Protocols

The *Points to Consider* (March 1, 1990, 55 FR 7447) under Part IV—Reporting Requirements states that:

"B. Reports regarding the general progress of patients should be filed with both your local IRB and ORDA within six-months of the commencement of the experiments and at six-month intervals thereafter. These twice-yearly reports should continue for a sufficient period of time to allow observation of all major effects. In the event of a patient's death, a summary of the special post-mortem studies and statements of the cause of death should be submitted to the IRB and ORDA, if available."

Dr. Brigid Leventhal, Chair of Working Group on Data Management,

will provide a summary of the reports submitted to ORDA by the principal investigators of NIH-approved protocols, and make recommendations regarding actions to be taken in the event of non-reporting.

XIX. Amendment to Section III-A-4 of the NIH Guidelines and the Points to Consider in the Design and Submission of Protocols for the Transfer of Recombinant DNA Into the Genome of Human Subjects Regarding the Term Subject(s)

During the March 1-2, 1993, Recombinant DNA Advisory Committee meeting, Dr. Robertson Parkman requested that the terms *subject* and *subjects* be amended to read *subject(s)*. Section III-A-4 currently reads:

"III-A-4. Deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into human subjects [21] * * *

Throughout the *Points to Consider* document the terms *subject* and *subjects* are used.

This amendment will clarify Section III-A-4 and the *Points to Consider*.

XX. Amendment to the Points to Consider in the Design and Submission of Protocols for the Transfer of Recombinant DNA Into the Genome of Human Subjects Regarding Satellite Institutions

On April 19, 1993, Dr. Nelson A. Wivel made a request that NIH-approved human gene therapy/gene transfer protocols may be conducted at satellite institutions with approval from the Chair of the Recombinant DNA Advisory Committee. The amendment would read:

"Protocols can be conducted at satellite institutions with approval from the Chair of the Recombinant DNA Advisory Committee. The Chair may consult others as necessary. The principal investigators must submit to the Office of the Recombinant DNA Activities the following: (1) Curricula vitae of new principal investigators, (2) Institutional Biosafety Committee approval, (3) Institutional Review Board approval, and (4) new Informed Consent document."

XXI. Amendment to the Points to Consider in the Design and Submission of Protocols for the Transfer of Recombinant DNA Into the Genome of Human Subjects Regarding Categorization of Protocols

The Working Group on Categorization of Protocols will make a report on decentralizing review for gene marking

protocols utilizing vectors that have been previously approved by the Recombinant DNA Advisory Committee.

XXII. Amendment to the Points to Consider in the Design and Submission of Protocols for the Transfer of Recombinant DNA Into the Genome of Human Subjects Regarding Protocol Format

The Working Group on Protocol Format will present its report on condensing materials required for the submission of human gene therapy/gene transfer protocols for review and approval by the Recombinant DNA Advisory Committee.

OMB's "Mandatory Information Requirements for Federal Assistance Program Announcements" (45 FR 39592, June 11, 1980) requires a statement concerning the official government programs contained in the *Catalog of Federal Domestic Assistance*. Normally, NIH lists in its announcements the number and title of affected individual programs for the guidance of the public. Because the guidance in this notice covers not only virtually every NIH program but also essentially every Federal research program in which DNA recombinant molecule techniques could be used, it has been determined not to be cost effective or in the public interest to attempt to list these programs. Such a list would likely require several additional pages. In addition, NIH could not be certain that every Federal program would be included as many Federal agencies, as well as private organizations, both national and international, have elected to follow the *NIH Guidelines*. In lieu of the individual program listing, NIH invites readers to direct questions to the information address above about whether individual programs listed in the *Catalog of Federal Domestic Assistance* are affected.

Dated: April 26, 1993.

Jay Moskowitz,

Deputy Director for Science Policy and Technology Transfer, NIH.

[FR Doc. 93-10486 Filed 5-3-93; 8:45 am]

BILLING CODE 4140-01-M

Study section	June-July 1993 meetings	Time	Location
Clinical Sciences-2, Mrs. Jo Pelham, Tel. 301-594-7254.	July 8-9	8:00	Residence Inn Marriott, Bethesda, MD.
Immunology, Virology & Pathology, Dr. Lynwood Jones, Tel. 301-594-7262.	July 14-16	8:30	Holiday Inn, Chevy Chase MD.
International & Cooperative Projects, Dr. G. B. Warren, Tel. 301-594-7289.	July 22-23	8:00	Embassy Suites Hotel, Chevy Chase Pavilion, Washington, DC.
Physiological Sciences, Dr. Nicholas Mazarella, Tel. 301-594-7098.	July 8-9	8:00	Holiday Inn, Crowne Plaza, Rockville, MD.

(Catalog of Federal Domestic Assistance Program Nos. 93.306, 93.333, 93.337, 93.393-93.396, 93.837-93.844, 93.846-93.878, 93.892, 93.893, National Institutes of Health, HHS)

Dated: May 24, 1993.

Susan K. Feldman,

Committee Management Officer, NIH.

[FR Doc. 93-12656 Filed 5-27-93; 8:45 am]

BILLING CODE 4140-01-M

Recombinant DNA Advisory Committee; Amend Notice of Meeting

Notice is hereby given to announce partial closure of the Recombinant DNA Advisory Committee meeting on June 7-8, 1993. The notice of meeting was published in the Federal Register on May 4, 1993 (58 FR 26676).

In accordance with the provisions set forth in section 552b(c)(6), title 5, U.S.C. and section 10(d) of Public Law 92-463, the meeting will be closed to the public on June 7 from approximately 5:40 p.m. to approximately 6:40 p.m., for the report and review of the expedited approval of a human gene transfer protocol submitted by Drs. Robert E. Sobol and Ivor Royston, including consideration of information of a personal nature, e.g., an individual's clinical records and findings, the disclosure of which would constitute a clearly unwarranted invasion of personal privacy.

Dated: May 19, 1993.

Susan K. Feldman,

Committee Management Officer, NIH.

[FR Doc. 93-12747 Filed 5-27-93; 8:45 am]

BILLING CODE 4140-01-M

Recombinant DNA Research: Proposed Actions Under the Guidelines—Correction

AGENCY: National Institutes of Health, PHS, DHHS.

ACTION: Notice of Proposed Actions Under the NIH Guidelines for Research Involving Recombinant DNA Molecules (51 FR 16958).

CORRECTION: On May 4, 1993 (58 FR 26676), the NIH published Proposed

Actions to be discussed during the upcoming meeting on June 7-8, 1993. The following additional item will be considered at that meeting:

XXIII. Report on Expedited Approval of Human Gene Transfer Protocol. In a letter dated April 30, 1993, Drs. Robert E. Sobol and Ivor Royston of the San Diego Regional Cancer Center, San Diego, California, requested the expedited review of a human gene transfer protocol. This request was for: (1) Additional treatment to a patient and (2) transduction of cells with a retrovirus vector, G1NaCvi2.

In accordance with the Procedures for Expedited Review of the National Institutes of Health (NIH) Guidelines for Research Involving Recombinant DNA Molecules (58 FR 21741), this request was reviewed by both intramural and extramural scientists. Drs. Royston and Sobol submitted additional documentation at the request of reviewers. Following evaluation of the additional data, the reviewers concluded that the preclinical and clinical information provided by the investigators was sufficient to justify continuation of the study with the new vector. The Director, NIH, approved Drs. Royston and Sobol's request on May 11, 1993.

The Procedures for Expedited Review, state in item number 7: "The NIH will report to the Recombinant DNA Advisory Committee (RAC) following expedited review and will include all of the materials on which the decision was based. The RAC will formally review the protocol at its next scheduled meeting. Patient privacy will be maintained." The RAC will review the expedited approval by the NIH Director at the June 7, 1993, meeting during a session that will be closed in order to protect patient privacy.

Dated: May 24, 1993.

Jay Moskowitz,

Deputy Director for Science Policy and Technology Transfer, NIH.

[FR Doc. 93-12748 Filed 5-27-93; 8:45 am]

BILLING CODE 4140-01-M

DEPARTMENT OF HOUSING AND URBAN DEVELOPMENT

Office of the Assistant Secretary for Community Planning and Development

[Docket No. N-93-1917; FR-3350-N-33]

Federal Property Suitable as Facilities To Assist the Homeless

AGENCY: Office of the Assistant Secretary for Community Planning and Development, HUD.

ACTION: Notice.

SUMMARY: This Notice identifies unutilized, underutilized, excess, and surplus Federal property reviewed by HUD for suitability for possible use to assist the homeless.

ADDRESSES: For further information, contact James N. Forsberg, room 7262, Department of Housing and Urban Development, 451 Seventh Street SW., Washington, DC 20410; telephone (202) 708-4300; TDD number for the hearing- and speech-impaired (202) 708-2565 (these telephone numbers are not toll-free), or call the toll-free title V information line at 1-800-927-7588.

SUPPLEMENTARY INFORMATION: In accordance with 56 FR 23789 (May 24, 1991) and section 501 of the Stewart B. McKinney Homeless Assistance Act (42 U.S.C. 11411), as amended, HUD is publishing this Notice to identify Federal buildings and other real property that HUD has reviewed for suitability for use to assist the homeless. The properties were reviewed using information provided to HUD by Federal landholding agencies regarding unutilized and underutilized buildings and real property controlled by such agencies or by GSA regarding its inventory of excess or surplus Federal property. This Notice is also published in order to comply with the December 12, 1988 Court Order in *National Coalition for the Homeless v. Veterans Administration*, No. 88-2503-OG (D.D.C.).

Properties reviewed are listed in this Notice according to the following categories: Suitable/available, suitable/

RECOMBINANT DNA ADVISORY COMMITTEE

Minutes of Meeting

June 7-8, 1993

U.S. DEPARTMENT OF HEALTH
AND HUMAN SERVICES
Public Health Service
National Institutes of Health

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**DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH
RECOMBINANT DNA ADVISORY COMMITTEE
MINUTES OF MEETING¹
June 7-8, 1993**

The Recombinant DNA Advisory Committee (RAC) was convened for its fifty-fourth meeting at 9:00 a.m. on June 7, 1993, at the National Institutes of Health (NIH), Building 31, Conference Room 6, 9000 Rockville Pike, Bethesda, Maryland 20892. Dr. LeRoy B. Walters (Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public except for the closed session to discuss the expedited approval of a human gene transfer protocol submitted by Drs. Ivor Royston and Robert Sobol which included confidential information. The following were present for all or part of the meeting:

Committee members:

Constance E. Brinckerhoff, Dartmouth Medical School
Alexander M. Capron, University of Southern California
Ira H. Carmen, University of Illinois
Gary A. Chase, Johns Hopkins University
Patricia A. DeLeon, University of Delaware
Roy H. Doi, University of California, Davis
Krishna R. Dronamraju, Foundation for Genetic Research
E. Peter Geiduschek, University of California, San Diego
Mariann Grossman, Hospital of the University of Pennsylvania
Robert Haselkorn, University of Chicago
Susan S. Hirano, University of Wisconsin
Donald J. Krogstad, Tulane University School of Medicine
Brigid G. Leventhal, Johns Hopkins Hospital
Abbey S. Meyers, National Organization for Rare Disorders
A. Dusty Miller, Fred Hutchinson Cancer Research Center
Arno G. Motulsky, University of Washington Medical School
Robertson Parkman, Childrens Hospital of Los Angeles
Leonard E. Post, Parke-Davis Pharmaceutical Division
Marian G. Secundy, Howard University College of Medicine
Brian R. Smith, Yale University School of Medicine

¹The RAC is advisory to the National Institutes of Health (NIH), and its recommendations should not be considered as final or accepted. The Office of Recombinant DNA Activities should be consulted for NIH policy on specific issues.

Stephen E. Straus, National Institutes of Health
LeRoy B. Walters, Kennedy Institute of Ethics, Georgetown University
Doris T. Zallen, VA Polytechnic Institute & State University

Executive secretary:

Nelson A. Wivel, National Institutes of Health
Debra J. Wilson, National Institutes of Health (Acting)

A committee roster is attached (Attachment I).

Non-Voting Agency Representative:

Kurt Gunter, Food and Drug Administration

National Institutes of Health staff:

Mohammad Amiri, NINDS
Leon Baltrucki, NHLBI
Norman Barton, NINDS
Alicia Bazzano, CC
R. Michael Blaese, NCI
Maritza Blanco, NINDS
Roscoe Brady, NINDS
Peter Bressler, NIAID
Steve Brody, NHLBI
Diane Bronzert, NCI
Jan Casadei, NCI
Chin Chu, NHLBI
Marinee Chuah, NHLBI
Ken Cowan, NCI
Kenneth Culver, NCI
Cindy Dunbar, NCI
Tony Eissa, NHLBI
MaryEllen Franko, NCI
Edward Ginns, NIMH
Marion Glick, NIAID
Barry Goldspiel, CC
Jeffrey Hoeg, NHLBI
Christine Ireland, OD
Ari Jaffe, NHLBI
Sachiko Kajigaya, NHLBI

Stefan Karlsson, NINDS
Masako Kawase, NHLBI
Becky Lawson, OD
Charles Link, NCI
Fred Lombardo, NHLBI
Catherine McKeon, NIDDK
Koichi Miyamura, NHLBI
Rubin Moreno, NCI
Craig Mullen, NCI
Pat Newman, NCI
Masahiro Ogasawara, NCI
Colette Parker, NINDS
Don Ralbovsky, OD
William Ramsey, NCI
Craig Reynolds, NCI
Yukio Sakiama, NCI
Nava Sarver, NIAID
Raffi Schiffmann, NINDS
Tom Shih, OD
Ellen Sidransky, NIMH
Toshihiro Soma, NHLBI
Stephanie Stahl, NINDS
Mary Sullivan, OD
Francis Taylor, NINDS
Thierry Vandendriessche, NHLBI
Keoki Williams, NCI
Matt Yeatman, NICHD

Others:

Paul Aebersold, Food and Drug Administration
French Anderson, University of Southern California
Nevin Andrews, National Narrowcast Network
Don Anthony, Case Western Reserve University
Alfred Bahnson, University of Pittsburgh
Arthur Bank, Columbia University
Jack Barber, Viagene, Inc.
John Barranger, University of Pittsburgh
James Barrett, Genetic Therapy, Inc.
Steve Bauer, Food and Drug Administration
William Benedict, MD Anderson Cancer Research Center
Bridget Binko, Cell Genesys, Inc.

John Bishop, Food and Drug Administration
G'dali Braverman, Act Up
Louis Bucalo, Ingenex, Inc.
Barrie Carter, Targeted Genetics, Inc.
Yawen Chiang, Genetic Therapy, Inc.
Edward Cohen, University of Illinois
Barbara Culliton, Nature Magazine
David Danar, Lehman Brothers
Timothy Darrow, Duke University
Tapas DasGupta, University of Illinois
Wanda de Vlaminck, Avigen, Inc.
Albert Deisseroth, MD Anderson Cancer Research Center
Michelle Durand, The French Embassy
Suzanne Epstein, Food and Drug Administration
Mason Essif, CNN
Susan Falen, Genetic Therapy, Inc.
Therese Falk, The Swedish Embassy
Ron Feldbaum, Financial Times
Mitchell Finer, Cell Genesys, Inc.
Bruce Fink, CNN
Bernie Fox, University of Michigan
Jeffrey Fox, BioTechnology
Ralph Freedman, MD Anderson Cancer Research Center
Siqing Fu, MD Anderson Cancer Research Center
Morgan Gale, Hearings-on-the-Line
J. David Gallagher, Barksdale Ballard
Jeffrey Galpin, Viagene, Inc.
Cyril Gay, Department of Agriculture
Lisa Giglio, The Pink Sheet
Richard Giles, University of Illinois
Robert Gilley, CNN
Steve Goff, Columbia University
T. Venkat Gopal, Clonexpress, Inc.
Cheryl Graham, Biometric Research Institute
Aileen Griffin, Viagene, Inc.
John Griffin, Viagene, Inc.
Janet Grun, Advanced Biosearch Associates
Elie Hanania, MD Anderson Cancer Research Center
Susanna Hegewisch, MD Anderson Cancer Research Center
Russell Herndon, Genzyme Corporation
Patrick Herve, CRTS Besancon, France
Charles Hesdorffer, Columbia Presbyterian Medical Center

Minoru Hirama, Viagene, Inc.
Sharon Hoff, Department of Agriculture
David Holzman, BioWorld
Joseph Ilan, Case Western Reserve University
John Jaugstetter, Genentech, Inc.
Susan Jenks, Journal of the National Cancer Institute
Joel Jessee, Life Technologies, Inc.
William Johnston, Baxter Health Care
Douglas Jolly, Viagene, Inc.
George Kalf, Thomas Jefferson University
Steve Kanzer, The Castle Group
Kathy Kaufmann, Theragen, Inc.
John Kavanagh, MD Anderson Cancer Research Center
Donald Kohn, Childrens Hospital of Los Angeles
Toshi Kotani, Genetic Therapy, Inc.
Alex Kuta, Food and Drug Administration
Donald Longnecker, Viagene, Inc.
Richard Lundberg, Biogen, Inc.
Daniel Maneval, Canji, Inc.
Carol Marcus-Sekura, Food and Drug Administration
Michael McCann, The Blue Sheet
Gerard McGarrity, Genetic Therapy, Inc.
Steven Mento, Viagene, Inc.
Bruce Merchant, Viagene, Inc.
Teri Merrill, Oncology News
Noel Messenger, Applied Immune Sciences
Fred Miller, Food and Drug Administration
Robert Moen, Genetic Therapy, Inc.
Richard Moscicki, Genzyme Corporation
Annemarie Moseley, Applied Immune Sciences
Gary Nabel, University of Michigan
Philip Noguchi, Food and Drug Administration
Terry O'Hanlon, Food and Drug Administration
Sheryl Osborne, Viagene, Inc.
Jeffrey Ostrove, Microbiological Associates, Inc.
Enzo Paoletti, Virogenetics Corporation
Stewart Parker, Targeted Genetics, Inc.
Liz Pennisi, Science News
Anne Petruska, The Blue Sheet
Cary Pfeffer, Biogen, Inc.
Stephen Pijar, University of Maryland
Chris Platsoucas, Temple University

Tom Porter, Theragen, Inc.
Raj Puri, Food and Drug Administration
Corey Raffel, Childrens Hospital of Los Angeles
Judy Randall, Economists Magazine
Robert Ratcheson, Case Western Reserve University
Michael Ravitch, Treatment Action Group
Rex Rhein, Biotechnology Newswatch
Jon Richards, University of Chicago
Paul Robbins, University of Pittsburgh
Renee Rockman, Genetic Therapy, Inc.
Igor Roninson, University of Illinois
H. John Roth, Department of Agriculture
Bruce Schackman, Furman Selz, Inc.
Alain Schreiber, Vical Inc.
Hilliard Seigler, Duke University
G. Terry Sharrer, Smithsonian Institution
Joseph Sherwin, Thomas Jefferson University
Tomiko Shimada, Ambience Awareness International
Thomas Storch, Tulane University Medical Center
Nevin Summers, Massachusetts Institute of Technology
Gary Temple, Life Technologies, Inc.
Pierre Tiberghien, CRTS Besancon, France
Paul Tolstoshev, Genetic Therapy, Inc.
Arvilla Trag, Virogenetics, Inc.
Mark Tykocinski, Case Western Reserve University
Christine Van de Pol, Rhone-Poulenc Rorer, Inc.
Trish Waitschies, MD Anderson Cancer Research Center
John Warner, Viagene, Inc.
Jody Waugh, The Novus Group
Julie Werner, The Novus Group
Clyde White, Carolina Biological Supply Co.
Hong-Ji Xu, Baylor College of Medicine

I. CALL TO ORDER

Dr. Walters (Chair) called the meeting to order and stated that notices of the meeting were published in the *Federal Register* on May 4, 1993 (58 FR 26676) and May 28, 1993 (58 FR 31045) as required by the *National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)*. He noted that a quorum was present and outlined the order in which speakers would be recognized. The primary and secondary reviewers will present their comments regarding the protocol, followed by responses from the principal investigators (PIs). The Chair will then recognize other committee members, *ad hoc* consultants, other NIH and Federal employees, the public who have submitted written statements prior to the meeting, followed by the public at large.

Dr. Walters thanked Dr. Wivel and his staff for handling a large volume of protocol materials and the RAC members for their timely review of the proposals. Dr. Walters reminded everyone to adhere to the schedule as outlined in the agenda. Dr. Wivel introduced a new staff member of the Office of Recombinant DNA Activities (ORDA), Dr. Thomas Y. Shih. Dr. Shih is a senior NIH research scientist who is well recognized for his work on *ras* oncogenes. His scientific background is most pertinent to his new position in ORDA.

II. MINUTES OF THE MARCH 1-2, 1993, MEETING

Dr. Walters called on Ms. Grossman to review the minutes of the March 1-2, 1993, RAC meeting. Ms. Grossman stated that the minutes were an accurate reflection of the March meeting. Minor corrections were submitted by Drs. Hirano and Miller. The RAC unanimously approved a motion made by Dr. Dronamraju and seconded by Dr. Smith to accept the March 1-2, 1993, RAC minutes, with the inclusion of minor grammatical changes, by a vote of 19 in favor, 0 opposed, and no abstentions.

III. DATA MANAGEMENT REPORT--REVIEW OF SEMIANNUAL REPORT FORMS FOR NIH-APPROVED HUMAN GENE TRANSFER PROTOCOLS/DR. LEVENTHAL

Dr. Leventhal summarized the semiannual report on RAC-approved human gene transfer protocols (Attachment II). The report period was for the 6 months prior to April 1, 1993. A total of 92 patients have been entered into the 36 RAC-approved protocols. The median time for these protocols to be activated was 8 months after the date of RAC approval. Of these 36 studies, 33 protocols are currently open. She noted that one of the report questions asking for evidence of gene expression was ambiguous. This question will be clarified to indicate that the PI must provide evidence of *in vivo* gene expression in the patient. *In vivo* evidence of gene transfer has been observed in 10 of the protocols; however, gene expression was not demonstrated in all cases. Two

possible adverse effects were reported. In one protocol, the patient's transplanted bone marrow failed to engraft; and in the other, a patient developed asymptomatic gliosis of the brain following treatment. It is unclear whether these two effects are related to gene transfer. Several patient deaths were reported; however, most of them were related to advanced cancer or complications arising from other clinical procedures. There is no evidence that any deaths have been directly related to the gene transfer procedures. Overall, the record on patient safety is reasonable.

Ms. Meyers raised two questions: (1) Why have fewer patients been accrued in these protocols than were approved by the RAC? (2) Why are there more patient deaths in the cancer protocols than the other studies? Dr. Leventhal explained that most of the protocols have been recently approved and are still open, which accounts for the apparent low accrual rate. Dr. Geiduschek suggested that the RAC consider implementing an expiration date for the protocol if no patients have been treated after a specified period of time. Regarding efficacy, Dr. Leventhal explained that most of the cancer protocols are Phase I trials for patients with large tumor burdens; therefore, demonstration of treatment efficacy is not expected. The report also specifically omitted the efficacy question based on the fact that full disclosure of research data might infringe on the ability of investigators to publish their results in peer-reviewed journals. Dr. Walters concurred with this policy. Dr. Parkman added that an interim statement of efficacy on a small number of patients is not statistically significant. The question of efficacy can be addressed in the renewal applications based on data from previous trials. In future data reports, published efficacy results will be included. Dr. Anderson stated that the June 1993 Data Management Report would be published in the journal *Human Gene Therapy*.

IV. MINOR MODIFICATIONS TO NIH-APPROVED HUMAN GENE TRANSFER PROTOCOLS

Dr. Walters summarized the minor modifications that have been approved to NIH-approved human gene transfer protocols. A total of 13 minor modifications have been approved, and are summarized in the form of a table (Attachment III). In response to Ms. Grossman's question on the requirements for a minor modification, Dr. Walters explained that a minor modification is a change that does not significantly alter the design of a protocol and that does not increase risk to the patient. A minor modification must be approved by the Institutional Biosafety Committee (IBC), Institutional Review Board (IRB), and the Chair of the RAC (in consultation with other RAC members as necessary).

V. **ADDITION TO APPENDIX D OF THE NIH GUIDELINES REGARDING A HUMAN GENE THERAPY PROTOCOL ENTITLED: *A PHASE I TRIAL OF HUMAN GAMMA INTERFERON-TRANSDUCED AUTOLOGOUS TUMOR CELLS IN PATIENTS WITH DISSEMINATED MALIGNANT MELANOMA*/DR. SEIGLER**

Review--Dr. Parkman

Dr. Walters called on Dr. Parkman to present his primary review of the protocol resubmitted by Dr. Hilliard F. Seigler of Duke University Medical Center, Durham, North Carolina. This protocol was deferred at the March 1993 RAC meeting. Dr. Parkman briefly recapitulated the protocol. This Phase I trial will administer human gamma interferon (γ -IFN)-transduced autologous tumor cells to patients with disseminated malignant melanoma. Autologous tumor cells will be grown in short-term culture and transduced with a vector expressing human γ -IFN. If these cells express a minimal level of γ -IFN, they will be irradiated with 10,000 rads and readministered to patients. The two endpoints to this study are: (1) clinical regression of tumors, and (2) the generation of cytotoxic T lymphocytes (CTL). Responding to previous concerns, the PI has provided data demonstrating that: (1) γ -IFN transduced cells induce increased cytolytic activity in peripheral blood, (2) the cytolytic activity is due to CTL as well as natural killer (NK) cell activity, (3) production of γ -IFN by transduced cells results in the up-regulation of Class I major histocompatibility (MHC) antigens, and (4) stimulation by transduced tumor cells results in increased CTL activity. Dr. Parkman stated that most of the scientific questions raised in the previous review have been addressed and recommended approval of the protocol.

Review--Dr. Leventhal

Dr. Leventhal stated that she was satisfied with the revised submission, and that the protocol should be approved in its present form.

Review--Ms. Meyers

Ms. Meyers raised a major concern about a statement in the Informed Consent document that requires patients to pay for any injury that occurs as a direct result of participation in the study. Dr. Carmen suggested minor changes to the Informed Consent document that would make the document more understandable to lay persons.

Investigator Response--Dr. Seigler

Dr. Seigler agreed to incorporate the minor changes in the Informed Consent document suggested by Dr. Carmen. In response to Ms. Meyers' concern about patient compensation, Dr. Seigler said that immediate care is available for any individual who is

injured as a direct result of their participation in research. The Informed Consent document informs patients that further information about their rights is available from the Hospital Risk Management Office.

Dr. Walters stated that compensation for research injuries is a generic issue that the RAC has discussed previously. On January 6, 1993, the RAC sent a letter addressing this issue to the NIH Director, Dr. Bernadine Healy. To date, no specific response to this letter has been received. There was a lengthy discussion on this issue, and Dr. Walters asked Dr. Parkman to draft a follow-up letter to the NIH Director that could be circulated for later discussion.

Committee Motion

A motion was made by Dr. Haselkorn and seconded by Dr. Parkman to approve the protocol. The motion passed by a vote of 20 in favor, 0 opposed, and no abstentions.

VI. ADDITION TO APPENDIX D OF THE NIH GUIDELINES REGARDING A HUMAN GENE THERAPY PROTOCOL ENTITLED: *USE OF MODIFIED RETROVIRUSES TO INTRODUCE CHEMOTHERAPY RESISTANCE SEQUENCES INTO NORMAL HEMATOPOIETIC CELLS FOR CHEMOPROTECTION DURING THE THERAPY OF OVARIAN CANCER: A PILOT TRIAL/DRS. DEISSEROTH, KAVANAGH, CHAMPLIN*

Review--Dr. Leventhal

Dr. Walters called on Dr. Leventhal to present her primary review of the protocol resubmitted by Drs. Albert B. Deisseroth, John Kavanagh, and Richard Champlin of the University of Texas M.D. Anderson Cancer Center, Houston, Texas. This protocol was deferred at the March 1993 RAC meeting. Dr. Leventhal explained that the investigators plan to introduce the cDNA of the multi-drug resistance gene (MDR-1) into normal hematopoietic early progenitor CD34(+) cells in patients with advanced ovarian cancer in an attempt to determine toxicity and the effect of modifying these normal stem cells. Responding to a question on the transduction procedure, the investigators have provided data demonstrating that human CD34(+) cells can be isolated and transduced, and have demonstrated adequate expression of MDR-1. The desired outcome will be that each successive incremental dose of Taxol will result in an increased percentage of MDR-positive bone marrow cells. She was concerned about the actual benefit these patients will derive from introducing the MDR-1 gene into their bone marrow stem cells. Adverse side effects of Taxol, such as neurotoxicity, will not be prevented by MDR-1 transduction; therefore, hematologic protection is the only possible beneficial outcome of this study. Dr. Leventhal stated that she is not completely satisfied that the experimental design of this protocol will provide a definitive answer about

hematological protection.

Review--Dr. Dronamraju

Dr. Dronamraju stated that Dr. Deisseroth had responded to his earlier concerns about the use of primate models. Dr. Deisseroth noted the RAC stated previously that either a large animal model or long-term bone marrow cultures were acceptable models, and that MDR-1 expression was demonstrated in bone marrow cells after 35 days in Dexter culture.

Review--Mr. Capron

Mr. Capron raised concerns about the Informed Consent document. Sections 3 and 4 of this document which describe the research plan and potential risks and benefits, are poorly written and very confusing. The description is too technical (e.g., using terms such as "pumping mechanism") rather than using simplified language (e.g., "a gene for drug resistance") to refer to the MDR-1 gene. The videotape prepared by the hospital is equally confusing.

Other Comments

Ms. Meyers said that the Informed Consent document may leave patients with the impression that the experimental treatment will cure their cancer. More conditionality should be incorporated into the document. She asked whether Dr. Deisseroth has obtained enough data from his previously approved protocols to justify approval of an additional protocol?

Dr. Leventhal responded that Dr. Deisseroth's two previously approved protocols are for chronic myelogenous leukemia, and that their progress is satisfactory. This protocol is for the treatment of ovarian cancer and is a well designed study.

Dr. Parkman questioned whether the administration of growth factor during hematopoietic recovery may obscure the clinical outcome of MDR-1 gene protection of Taxol toxicity to bone marrow cells. MDR gene expression will be monitored by polymerase chain reaction (PCR) analysis of colonies derived from peripheral blood and bone marrow cells following Taxol treatment. An increase in the number of MDR-1(+) colonies following Taxol administration will be a definitive endpoint, even if any protective effects due to MDR-1 expression are normalized by hematopoietic cell growth factor administration. Furthermore, if MDR-1 transduction does not result in clinical benefit because of Taxol toxicity to cells other than the bone marrow cells, the proposed study will still provide useful information for future attempts to convey drug resistance to hematopoietic cells using other genes which may have greater therapeutic margins.

Dr. Chase stated that he primarily considers safety and the responses to the *Points to Consider in the Design and Submission of Protocols for the Transfer of Recombinant DNA into the Genome of Human Subjects (Points to Consider)* when reviewing human gene transfer protocols, not necessarily that the best experiment has been proposed. Dr. Walters added that the RAC should make a threshold judgment that there is a reasonable hope of success. Dr. Leventhal agreed that it is not ethical to allow a patient to participate in an experiment that is so poorly designed that no useful information will be obtained.

Dr. Carmen suggested simplified language that would make the Informed Consent document more understandable to lay persons. Drs. Post and Smith asked the investigators to clarify the bone marrow culture and transduction procedures. Dr. Geiduschek raised a concern about the adequacy of testing a small fraction of the vector supernatant for replication-competent retrovirus (RCR), and objected to the use of the term "safety modified viruses" throughout the Informed Consent document and in the protocol title. Dr. Walters recommended that the word "stem" should be omitted from the titles of both the protocol and the Informed Consent document.

Investigator Response--Dr. Deisseroth

Dr. Deisseroth explained that he would limit his response to the issue of MDR-1 expression in hematopoietic cells followed by specific questions raised by the RAC members. He presented data demonstrating that MDR-1 transduced CD34(+) cells "pump out" the control rhodamine dye more efficiently than untransduced CD34(+) cells. Since these transduced cells express a higher level of MDR-1 gene product, protection against Taxol toxicity is conferred. Expression of mRNA by transduced cells was detected by PCR analysis. The transduced gene was distinguishable from the endogenous cellular gene by a point mutation.

Dr. Deisseroth described the schema for the proposed clinical protocol. Following collection of peripheral blood and bone marrow cell for transduction, a conditioning regimen of thiotepa-cyclophosphamide will be delivered to reduce the total body tumor burden and to eradicate the bone marrow. Following autologous bone marrow (ABM) transplantation, a fraction of which has been transduced with the MDR-1 gene, patients will undergo a period of recovery prior to Taxol treatment. He stated that in the clinical protocol that does not involve gene transfer, only 1 out of 30 patients who received the conditioning regimen experienced mild marrow failure. Responding to questions about possible toxicity associated with retrovirus transduction of ABM cells, he cited the retrovirus transduction marking data obtained from studies conducted both at MD Anderson and St. Jude demonstrates that no difference in engraftment frequency has been observed by 30 days in patients receiving either marked or unmarked ABM cells.

Dr. Deisseroth stated that patients have received granulocyte colony stimulating factor (G-CSF) as part of the Taxol treatment regimen. Therefore, G-CSF will be administered to patients receiving transduced ABM cells to allow direct comparison of results. Since patients receiving G-CSF still exhibit a certain degree of hematopoietic toxicity due to Taxol, any protective effect of MDR-1 expression will be detectable. There is a dose window in which Taxol toxicity primarily involves the bone marrow and not the neural or gastrointestinal systems; therefore, bone marrow protection can be demonstrated. Long-term protection of the bone marrow from Taxol toxicity has been demonstrated in the murine model using the same vector proposed for the human study. Responding to questions raised by Drs. Post and Smith about the stromal cells used to enhance the transduction efficiency of the CD34(+) cells, Dr. Deisseroth stated that the monolayer stromal cells are grown from a standard diagnostic bone marrow aspirate performed 2 weeks prior to the transduction procedure. In response to Dr. Geiduschek's question about the adequacy of RCR safety testing, Dr. Deisseroth stated that many safety tests have been performed, but that all of the data has not been prepared for presentation at today's meeting.

Committee Motion

A motion was made by Dr. Leventhal and seconded by Dr. Dronamraju to approve this protocol contingent on submission of the following: (1) data on the pre- and post-production RCR testing, and (2) a revised Informed Consent document that incorporates the changes suggested by Mr. Capron, Ms. Meyers, Dr. Leventhal and Dr. Walters. The motion passed by a vote of 21 in favor, 0 opposed, and no abstentions.

VII. AMENDMENT TO THE HUMAN GENE THERAPY PROTOCOL ENTITLED: *TREATMENT OF SEVERE COMBINED IMMUNE DEFICIENCY (SCID) DUE TO ADENOSINE DEAMINASE (ADA) DEFICIENCY WITH CD34(+) SELECTED AUTOLOGOUS HEMATOPOIETIC STEM CELLS/DR. BLAESE*

Review--Dr. Post

Dr. Walters called on Dr. Post to present his primary review of the minor modification submitted by Drs. R. Michael Blaese and Craig A. Mullen of the NIH, Bethesda, Maryland. Dr. Post stated that this modification represents the following significant changes to the previously approved protocol: (1) an additional source of cells, i.e., cord blood and placenta, (2) an expanded eligibility criterion that includes newborn babies, and (3) the administration of transduced CD34(+) cells alone, and (4) the inclusion of additional sites at which the gene therapy procedure will be performed. This modification would have been more appropriately handled as an expedited review protocol; however, the expedited review procedures were not yet officially incorporated into the *NIH Guidelines*. He recommended approval of this minor modification based

on: (1) the imminent birth of the newborns, (2) the gene therapy safety issues are the same as the original protocol, and (3) the prospective benefit to these infants.

Review--Dr. Smith

Dr. Smith agreed with the issues discussed by Dr. Post. Dr. Smith recommended the RAC should approve the proposed minor modification.

Review--Dr. Leventhal

Dr. Leventhal recommended that the proposed minor modification should be approved contingent on the following: (1) the diagnosis will be confirmed at birth, (2) there will be no maternal contamination of the cord blood, (3) polyethylene glycol (PEG)-ADA will be administered, and (4) a portion of the cord blood will be cryopreserved.

Review--Dr. Walters

Dr. Walters stated that the investigators have outlined the differences between the proposed minor modification and the major amendment that was approved by the RAC at its February 1992 meeting: (1) G-CSF will not be administered to the newborns, (2) leukapheresis will not be performed to harvest CD34(+) cells, and (3) the requirement for PEG-ADA pretreatment has been omitted. He asked the investigators to respond to the following questions: (1) Will PEG-ADA be withdrawn at the discretion of the PI? (2) Is bone marrow aspiration in newborns safe and necessary? (3) Are there any preclinical studies in which newborn animals have received autologous transduced cells obtained from cord blood or placenta? (4) At what point in the pregnancy is fetal ADA deficiency detected? Are there alternative therapies? (5) Will a sufficient supply of a new stem cell growth factor be available for these patients to be treated in May 1993? and (6) Will the LASN or G1NaSvADA vector be used to treat these infants?

Other Comments

Ms. Meyers questioned whether there are separate Informed Consent documents for patients that will be treated at the additional sites outside of the NIH. Dr. Doi asked whether transduced cryopreserved CD34(+) cells have been demonstrated to be as effective as transduced fresh cells. In response to Dr. Kohn's statement that the Food and Drug Administration (FDA) has determined that the current lot of LASN vector is unacceptable for clinical use based on new RCR testing standards, Dr. Post inquired about the accuracy of this statement.

Ms. Grossman suggested that the proposed revision is more than a minor modification and should have been treated as an expedited review protocol. She objected to the

breach of patient confidentiality in this case and was concerned that the current publicity will impact on patient enrollment in other gene therapy protocols. Dr. Walters explained that this request came on April 13, 1993, and that the expedited review procedures were not officially in effect at that time. Therefore, the revision was treated as a minor modification.

Investigators' Responses--Drs. Blaese and Kohn

Responding to Dr. Post's question, Dr. Blaese explained that the FDA has determined that the previous lot of LASN may be used only for patients on the existing protocol and will not be permitted for use in this amended protocol. Subsequently, a new production lot of LASN has been produced and will be available for use on the two newborns. Regarding Dr. Doi's question, Dr. Blaese said that fresh CD34(+) cells will be used immediately without cryopreservation. In response to the confidentiality issue raised by Ms. Grossman, Dr. Blaese stated that patient confidentiality was maintained almost 3 years for the initial ADA patients. Ultimately, the parents chose to publicize the encouraging results that are being observed in their children. In the case of these newborns, the parents voluntarily waived their confidentiality and encouraged press contact. Ms. Meyers expressed her concern on patients' families going public which could affect their ability to make proper informed consent. Drs. Miller and Leventhal agreed on the validity of Ms. Meyers' concern; however, this issue should not be the basis for disapproval of this minor modification. In regard to Dr. Walters' question about the time of diagnosis, Dr. Blaese said that these two families have previous children born with ADA deficiency; therefore, the mothers were monitored for the defect. Following a positive diagnosis, the families expressed interest in pursuing stem cell gene therapy using cord blood and placenta. Regarding Ms. Meyers's question about Informed Consent documents, Dr. Blaese said that these documents have been prepared for each of the collaborating institutions. Ms. Meyers commented that all of these documents were not submitted for RAC review. Dr. Leventhal stated that if the additional documents are essentially the same as the one approved by the RAC, then they would be acceptable.

Dr. Leventhal suggested that the withdrawal of PEG-ADA should be at the discretion of the PIs since they possess the expertise to make such a decision. She noted that bone marrow aspiration is a routine procedure for 6 month old children; therefore, the procedure is acceptable if properly described in the Informed Consent document. Dr. Kohn responded that the aspiration procedure is clearly stated in the Informed Consent document, and that the patients' parents have the right to refuse the procedure. Dr. Motulsky concurred with Dr. Leventhal's comments.

Dr. Kohn stated that only fresh cord blood will be used for transduction and reconstitution of stem cells because the effects of cryopreservation are unknown. The

necessity for fresh cord blood is the basis for this minor modification.

Committee Motion

A motion was made by Dr. Post and seconded by Dr. Motulsky to approve the minor modification allowing for the treatment of ADA-deficient newborns with autologous CD34(+) cells obtained from the cord blood and placenta. Also, this modification will allow the PIs to: (1) withdraw PEG-ADA at their discretion, and (2) perform bone marrow aspirations, as necessary, to monitor for gene transduction. The motion passed by a vote of 18 in favor, 0 opposed, and 3 abstentions.

VIII. ADDITION TO APPENDIX D OF THE NIH GUIDELINES REGARDING A HUMAN GENE THERAPY PROTOCOL ENTITLED: *IMMUNOTHERAPY FOR CANCER BY DIRECT GENE TRANSFER INTO TUMORS/DR. NABEL*

Review--Dr. Doi

Dr. Walters called on Dr. Doi to present his primary review of the protocol submitted by Dr. Gary J. Nabel of the University of Michigan Medical Center, Ann Arbor, Michigan. This protocol is an extension of the study approved by the RAC at its February 1992 meeting. The goal of this protocol is to improve the efficacy of tumor immunotherapy by the introduction of a gene encoding a foreign Class I MHC protein. The investigators propose that enhanced production of this protein will augment a CTL response against unmodified tumor cells. This protocol incorporates modifications that were not part of the original protocol approved by the RAC. These changes include: (1) the use of more efficacious cationic liposomes in which 1,2-dimyristyloxypropyl-3-dimethyl-hydroxyethylammoniumbromide (DMRIE) is utilized with dioleoyl-phosphatidylethanolamine (DOPE) for more efficient delivery of the gene; (2) the use of an improved vector, which includes the $\beta 2$ microglobulin gene that forms a complete complex with Class I MHC gene product; (3) the inclusion of a catheter-based gene delivery system for direct gene transfer into the tumor microcirculation; and (4) application of the system to different tumor types. Preliminary murine data indicate that the new DMRIE/DOPE liposome preparation: (1) improves transfection efficiency, (2) demonstrates minimal toxicity, and (3) enhances the anti-tumor effect against the foreign MHC gene. Dr. Doi posed the following questions: (1) Has liposome transfection proved to be safe and efficacious in the previous RAC-approved protocol? (2) Have anti-tumor effects been observed? (3) Have all of the previous safety and toxicity concerns been satisfactorily resolved? (4) Will the increased transduction efficiency *in vitro* with DMRIE/DOPE be reproducible *in vivo*? (5) Are there any possible adverse effects on the immune response to DMRIE/DOPE? (6) How precise is the catheter delivery? (7) Will normal cells be transduced? and (8) What is the discomfort level in patients undergoing catheter administration? If the investigators can satisfactorily

respond to these questions, he would recommend approval of this protocol.

Review--Dr. Brinckerhoff

Dr. Brinckerhoff agreed with Dr. Doi's assessment of the protocol. She noted that the investigators have not provided an extensive summary of previous results. The PI should explain whether the large doses of liposome that will be administered by catheter to the patients is safe. She stated that the investigators adequately responded to her written comments and that she recommends approval of the protocol.

Review--Dr. Secundy (Presented by Dr. Brinckerhoff)

In Dr. Secundy's absence, Dr. Brinckerhoff summarized her written review. Dr. Secundy states that in the Informed Consent document should be clarified and written in simplified language. In addition, the *Alternative Therapies* section should be clarified.

Other Comments

Ms. Grossman asked whether this protocol is an extension or a replacement of the previous study, and a description of the types of tumors that will be treated. Dr. Carmen asked whether the IL-2 that will be administered refers to the gene or the gene product. Ms. Meyers suggested that the Informed Consent document should recommend male and female contraception and include a request for autopsy. Dr. Miller stated that the vector sequence was checked through GenBank, and that there are no harmful sequences or open reading frames that pose any concern. Dr. Geiduschek asked the PI to comment on the comparative merit of liposome delivery versus retrovirus vector in regard to technical issues such as transduction efficiency, mitotic state of target cells, etc. Dr. Krogstad asked the PI to elaborate on the animal data.

Investigator Response--Dr. Nabel

Dr. Nabel explained that this study is a new protocol, not the extension of the previous protocol. Intratumoral injection of liposome complexes is currently used in the treatment of melanoma. When catheter-based delivery is employed, other tumor types will be targeted that have a well-defined vascular blood supply, e.g., hepatic tumors. Animal studies indicate that DMRIE/DOPE is more efficacious and less toxic than the liposome preparation used in the previous trial. There have been no adverse effects associated with the old liposome preparations. The new liposome-foreign MHC gene preparation provides protection in animals. At 1,000-fold higher doses of the new preparation, no toxicity is observed. He explained that much higher doses of the new vector will be obtainable.

Dr. Nabel summarized previous results from 5 melanoma patients. The clinical

endpoints used to evaluate the trial were: (1) gene expression, (2) toxicity, and (3) antitumor response. In 4 of 5 patients, RNA expression of the injected DNA was detected by reverse transcriptase PCR on cells obtained at the site of injection. Protein expression was assayed by immunofluorescence and immunostaining techniques in several patients. Generally, there was no toxicity regarding renal, myocardial, liver, and hematological functions. The anti-DNA immune response did not increase following DNA-liposome treatment; therefore, the primary endpoint of the trial has been achieved. CTL responses to autologous tumor, particularly against human leukocyte antigen (HLA)-B7, were observed. In at least one patient, a melanoma nodule disappeared 85 days following treatment. Several other tumor responses were observed; however, these responses could be non-specific. Dr. Nabel stated that he is encouraged by the augmented CTL responses to the injected gene product.

Discussion

Drs. Parkman and Miller expressed satisfaction in the initial trial. Dr. Nabel cautioned that only 1 patient demonstrated a significant antitumor response to the treatment. The new liposome preparation will allow delivery of 100-fold more DNA. Therefore, the proposed study may produce more efficacious results. Regarding the animal studies, the antitumor response appears to be dependent on the gene that is injected rather than the non-specific response to liposome-DNA complexes. In response to Dr. Carmen's question about IL-2, Dr. Nabel explained that patients will receive the IL-2 protein not the gene.

Dr. Nabel explained the rationale for including other tumor types. Melanoma was chosen for the initial studies because noninvasive treatment was required. In the proposed protocol, other tumor types will be treated; 12 patients will be treated by direct intratumor injection and 12 patients by the catheter delivery method. The latter will be administered to patients with colon cancer, renal cell cancer, and metastatic melanoma. Renal and liver tumors with well accessible blood supplies are preferable targets for this therapy. Following a lengthy discussion about the types of tumor that should be approved for treatment, the RAC agreed not to place any restrictions on the tumor type. The committee members recommended that patients should have a good performance status in order to ensure the likelihood of successful treatment.

Committee Motion

A motion was made by Dr. Miller and seconded by Dr. Doi to approve this protocol contingent on submission of the following: (1) a revised patient eligibility section including the provision that patients who are eligible for catheter delivery of cationic liposomes must have a performance status of 0-1, and (2) a revised Informed Consent document that includes a recommendation that male/female patients use contraception

and that females not be pregnant or plan to become pregnant while participating in the study, an explanation of long-term follow-up, and a request for autopsy. The motion passed by a vote of 18 in favor, 0 opposed, and 2 abstentions.

IX. WORKING GROUP REPORT ON SUBMISSION OF HUMAN GENE TRANSFER PROTOCOLS/DR. BRINCKERHOFF

Dr. Walters called on Dr. Brinckerhoff to present the report on the Working Group on the Submission of Protocols. The working group consisted of Drs. Brinckerhoff, Miller, Krogstad, and Ms. Meyers. A telephone conference call was held on May 28, 1993. The working group discussed whether investigators submitting human gene transfer protocols for RAC review should continue to be required to submit both the clinical protocol and the *Points to Consider* as part of their submission. Due to a substantial increase in the number of protocols submitted for RAC review, many committee members expressed concern about the volume of paperwork that is required; stating that the submission materials have reached an unmanageable level.

Committee motion

A motion was made by Dr. Chase and seconded by Dr. Krogstad to endorse the recommendations of the Working Group on Submission of Human Gene Transfer Protocols and to amend the *Points to Consider* of the *NIH Guidelines* as appropriate. The motion passed by a vote of 22 in favor, 0 opposed, and no abstentions. The recommendations of the working group are as follows:

1. The RAC should more strictly enforce the page limits for the protocol (20 pages) and *Points to Consider* (4-5 pages) as required by the *Guidelines for the Submission and Review of Human Gene Transfer Protocols by the RAC* of the *Points to Consider*. References may be made to supplemental materials. Discretion should be used on the part of investigators about the amount of supplemental materials that are included in their submissions. Materials will not be accepted if typed in a font that is more than 15 characters per inch.
2. The *Points to Consider* should remain a free standing document because protocols are not always succinct. The *Points to Consider* assists investigators in focusing their answers to specific questions. The investigators should provide complete responses to each question and if necessary, specifically refer the reviewers to portions of the protocol that address certain issues more fully.
3. The investigators should focus on following the Informed Consent Section of the *Points to Consider*. The working group will develop a guidance document for investigators to use in developing appropriate Informed Consent documents.

X. ADDITION TO APPENDIX D OF THE NIH GUIDELINES REGARDING A HUMAN GENE THERAPY PROTOCOL ENTITLED: *GENE THERAPY FOR GAUCHER DISEASE: EX VIVO GENE TRANSFER AND AUTOLOGOUS TRANSPLANTATION OF CD34(+) CELLS*/DR. BARRANGER

Review--Dr. Haselkorn

Dr. Walters called on Dr. Haselkorn to present his primary review of the protocol submitted by Dr. John A. Barranger of the University of Pittsburgh, Pittsburgh, Pennsylvania. Dr. Haselkorn stated there are two protocols being presented at this RAC meeting for the treatment of Gaucher disease; therefore, there are several general comments that apply to both protocols. Gaucher disease is an ideal genetic disorder for gene therapy. It is likely that positive results could be obtained similar to those reported for the ADA protocol, with the added feature that Gaucher disease is a much more prevalent disease. Gaucher disease results from an accumulation of glucocerebroside in macrophages due to a deficiency in the glucocerebrosidase (GC) enzyme. Type I is the most prevalent form of Gaucher disease resulting in less than 25% of the normal level of enzyme activity in homozygotes. The rarer forms of the disease, Types II and III, also involve the central nervous system and can result in early death. Current therapy involves purified GC enzyme; however, this treatment is extremely expensive which justifies gene therapy as a viable alternative.

Dr. Haselkorn explained that in this proposal G-CSF will be used to mobilize CD34(+) cells in the peripheral blood. In turn, these stem cells will be harvested and transduced with retrovirus vectors encoding the absent GC enzyme. The hypothesis is that GC will enter the macrophages and alleviate the lipid storage condition. The investigators propose to treat the first two patients without bone marrow ablation. If these two patients engraft without prior ablation, the remaining patients will be treated similarly. If these two patients do not engraft, then the remaining patients will receive low doses of Cytoxan to produce partial myeloablation. He asked questions concerning the choice of cells, the collection and transduction of cells, the level of gene expression, the necessity of marrow ablation, and how the two different vectors proposed for the two different Gaucher disease protocols compare.

Review--Ms. Grossman

Ms. Grossman stated that the investigators have submitted inadequate data demonstrating GC expression enzyme using the proposed vector in the human target cells. She questioned whether the transduced cells will engraft without myeloablation. If myeloablation is necessary, does the potential risk justify the use of gene therapy since an effective alternative therapy is available?

Review--Dr. Carmen

Dr. Carmen stated that Gaucher disease appears to be a paradigm affliction made to order for gene therapy. He recognized the similarity between this disease and ADA deficiency in which the transduced cells have been observed to have growth advantage in engrafted bone marrow, therefore, minimizing the need for myeloablation. Data suggest that even a low level of GC enzyme expression may be sufficient to ameliorate the condition. He recommended that the protocol be approved.

Other Comments

Dr. Smith expressed concern about the need for myeloablation and questioned whether Cytoxan is an adequate ablative regimen for bone marrow transplantation. Dr. Leventhal commented that patients receiving transduced CD34(+) cells without myeloablation will have other treatment options if engraftment is not successful.

In response to Ms. Grossman's critique, Dr. Motulsky stated that as a medical geneticist, he is not in agreement with her comments about the validity of Gaucher disease as a candidate for gene therapy. Gaucher disease is an ideal candidate for gene therapy since enzyme replacement is expensive and the treatment has to be continually repeated. Ms. Meyers said that the Informed Consent document should include the following: (1) a suggestion that contraception should be used by males and females, and (2) a request for autopsy. Ms. Meyers questioned why patients under 18 years old are excluded from participation in this study.

Responding to Dr. Haselkorn's question, Dr. Miller stated that the two vectors proposed for the two Gaucher protocols are functionally identical. Both vectors are based on the Moloney murine leukemia virus, and produce only the GC enzyme without other vector proteins including *gag*. Dr. Haselkorn suggested that additional *in vitro* experiments should be submitted in which the two Gaucher vectors are directly compared in human hematopoietic cells. Dr. Krogstad expressed concern that an immune response might be generated against the GC enzyme. Dr. Parkman commented that most patients will be tolerant to gene expression although there have been a few reported instances of antibody responses against the recombinant enzyme. Dr. Parkman noted that the recombinant enzyme differs from the cellular enzyme in protein glycosylation.

Investigator Response--Dr. Barranger

Responding to the question of myeloablation, Dr. Barranger explained that the first two patients will be treated with genetically corrected CD34(+) cells obtained from peripheral blood to assess whether significant engraftment is achieved without myeloablation. GC enzyme activity will be measured in the peripheral blood leukocytes

to assess the success of engraftment. Animal studies indicate that a small correction of functional GC enzyme activity results in a therapeutic response. Dr. Smith asked whether the results obtained from two patients will conclusively indicate whether myeloablation is necessary for the remaining patients. Dr. Barranger responded that the first two patients will be evaluated after the first transplantation with CD34(+) cells; and if necessary, the procedure will be repeated until sufficient GC expression is demonstrated. Animal experiments demonstrate engraftment without myeloablative therapy. Dr. Barranger stated that he plans to treat patients without myeloablation. Several RAC members questioned whether this stopping rule is explicitly written in the protocol. Dr. Barranger clarified the flow sheet in the protocol and said that if the first two patients succeed in the transplant, then the remaining patients will not receive myeloablation.

Responding to Ms. Meyers' question about the exclusion of children, Dr. Barranger said that it is desirable to obtain results in adult patients first before considering the treatment of children. Dr. Barranger agreed to revise the Informed Consent document regarding contraception for men and women, a request for autopsy, and other minor changes suggested by Ms. Meyers.

Dr. Barranger stated that some patients will receive GC enzyme replacement therapy. The exogenous enzyme will not interfere with measurement of the gene-expressed enzyme level in the peripheral blood. He agreed to include a statement in the protocol explaining that the enzyme therapy will not be terminated until engraftment has been demonstrated. Dr. Krogstad asked whether anti-GC antibodies will react with the enzyme produced by genetically corrected leukocytes? Dr. Barranger responded that this issue has not been addressed and agreed to revise the protocol to exclude patients who have anti-GC antibodies. Dr. Miller was concerned that if myeloablation is to be considered for this protocol, large animal experiments should be performed. Dr. Smith suggested that the protocol should be amended to eliminate the myeloablation procedure. The PI should resubmit a request for RAC review if myeloablation is to be considered. Dr. Barranger agreed to this stipulation.

Committee Motion

A motion was made by Dr. Haselkorn and seconded by Dr. Motulsky to approve the protocol with the following stipulations: (1) a maximum of 5 patients will be entered onto the study, (2) patients will not receive cyclophosphamide ablation of bone marrow, (3) patients who demonstrate antibodies against GC will not be eligible for the protocol, (4) GC enzyme replacement therapy will not be discontinued until cytochemical evidence of engraftment is demonstrated, and (5) the Informed Consent document will be revised to include a recommendation that male/female patients use contraception and females not be pregnant or plan to become pregnant while participating in the study, an

explanation of long-term follow-up, a request for autopsy in the event of death, protection from the media, and a statement informing patients that although they may receive no direct benefit from the protocol, knowledge may be gained that will benefit others. The motion passed by a vote of 15 in favor, 0 opposed, and 4 abstentions.

XI. ADDITION TO APPENDIX D OF THE NIH GUIDELINES REGARDING A HUMAN GENE THERAPY PROTOCOL ENTITLED: *RETROVIRAL MEDIATED TRANSFER OF THE cDNA FOR HUMAN GLUCOCEREBROSIDASE INTO HEMATOPOIETIC STEM CELLS OF PATIENTS WITH GAUCHER DISEASE*/DRS. KARLSSON, DUNBAR AND KOHN

Review--Dr. Haselkorn

Dr. Walters called on Dr. Haselkorn to present his primary review of the protocol submitted by Drs. Stefan Karlsson and Cynthia Dunbar of the NIH, Bethesda, Maryland, and Dr. Donald B. Kohn of Childrens Hospital of Los Angeles, Los Angeles, California. Dr. Haselkorn stated that his general comments regarding gene therapy for Gaucher disease that he provided during the review of Dr. Barranger's protocol are also applicable to this protocol. This protocol has the potential to produce results as dramatic as those observed in the ADA gene therapy protocol. One major concern regarding this protocol is that it involves two institutions, the NIH and Childrens Hospital of Los Angeles. Two different protocols are described, one using bone marrow cells and the other using peripheral blood cells. Although the protocol is for 10 patients, it is unclear how many patients will be treated at each institution and how the choice of target cells to be transduced will be determined. Dr. Haselkorn stated his concern that the *in vivo* enzyme levels are not very significant with the proposed vector. Enzyme levels were significantly higher using other vector constructs. Dr. Haselkorn stated that all of his other concerns have been adequately addressed by the PIs.

Review--Dr. Motulsky

Dr. Motulsky stated that Gaucher disease is a recessive disease that affects Jewish people of European origin with a substantial frequency of 1 in 500. There is tremendous variability in the clinical manifestations of this disease. The correlation between glucocerebrosidase (GC) enzyme activity and clinical severity is not predictable. Even with the most common mutation of Type I Gaucher disease (nucleotide 1226), there is considerable variation in clinical severity. How will this variability affect patient selection? Will previous enzyme therapy interfere with determining the success of the gene therapy procedure? The investigators have explained that GC expression by transduced cells can be distinguished from the activity of the exogenous enzyme. The other concerns about preclinical studies and CD34(+) cell transplantation without myeloablation were adequately addressed by the PI. Several specific minor suggestions

in the Informed Consent documents were outlined. Overall, the protocol is well thought out and promises to be successful. Dr. Motulsky recommended approval of the protocol with the inclusion of minor revisions.

Review--Dr. Carmen

Dr. Carmen stated that animal data demonstrated that bone marrow cell ablation is not a necessary predicator for correcting enzyme deficiency. The protocol will test this hypothesis in humans. The research design is lucid and carefully crafted. The Informed Consent document correctly emphasizes the gene "transfer" rather than the "therapy" features of the project. Dr. Carmen asked the PI to explain why Childrens Hospital of Los Angeles will perform transduction of bone marrow and the NIH will transduce peripheral blood cells. Why is the vector proposed for the human study different from the vector used in preclinical studies? Dr. Carmen recommended approval of the protocol.

Investigator Response--Dr. Karlsson

Responding to Dr. Haselkorn's question regarding the choice of vector, Dr. Karlsson explained that G1Gc was chosen as the vector because it produced the highest titers and levels of gene expression by macrophages in the murine model. When the MSG vector system became available later, no additional advantage was found. Since there is little human experience with CD34(+) cell transduction and transplantation without myeloablation, a comparison will be made between bone marrow cells and peripheral blood cells. Five patients will receive transduced bone marrow cells, and 5 patients will receive transduced peripheral blood cells. Investigators at the NIH have extensive experience with peripheral blood cells, and investigators at the Childrens Hospital of Los Angeles possess expertise with ABM transplantation. If one cell source proves to be more efficacious than the other, the optimal source will be used by both institutions. Responding to Dr. Carmen's comment about the choice of PA317/G1Gc vector and packaging cell line, Dr. Karlsson stated that this packing cell line and vector is probably functionally equivalent to Dr. Barranger's packaging cell line and vector. The PA317/G1Gc system is available and efficacious based on transduction and expression data in the target CD34(+) cells. Dr. Kohn agreed with Dr. Karlsson's statement regarding the similarity between the two vectors. Ms. Meyers commented that the Informed Consent document is very well written.

Committee Motion

A motion was made by Dr. Motulsky and seconded by Dr. Haselkorn to approve the protocol. The motion passed by a vote of 14 in favor, 0 opposed, and 4 abstentions.

XII. ADDITION TO APPENDIX D OF THE NIH GUIDELINES REGARDING A HUMAN GENE THERAPY PROTOCOL ENTITLED: *A PRELIMINARY STUDY TO EVALUATE THE SAFETY AND BIOLOGIC EFFECTS OF MURINE RETROVIRAL VECTOR ENCODING HIV-1 GENES [HIV-IT(V)] IN ASYMPTOMATIC SUBJECTS INFECTED WITH HIV-1/DRS. GALPIN AND CASCIATO*

Review--Dr. Hirano

Dr. Walters called on Dr. Hirano to present her primary review of the protocol submitted by Drs. Jeffrey E. Galpin of the University of Southern California, Los Angeles, California, and Dennis A. Casciato of the University of California, Los Angeles, California (sponsored by Viagene, Inc., San Diego, California). The overall goal of this study is to evaluate the use of retrovirus vector-mediated gene transfer for the treatment of human immunodeficiency virus (HIV)-infected individuals. The N2 retrovirus vector carrying *env/rev* gene of HIV-1 will be administered to asymptomatic HIV infected individuals. The hypothesis is that the vector will express the HIV *env* protein within cells and induce an augmented CTL response. This augmented response may slow or reverse disease progression. The safety and biological effects of the vector have been assessed in mice and in nonhuman primates. These *in vivo* studies demonstrated the lack of any acute or chronic toxicity and induction of enhanced CTL and antibody responses. The specific objective of this protocol is to evaluate these parameters in human subjects. Intramuscular injections will be administered monthly for 3 months and patients will be followed for evidence of virus replication, HIV burden, and HIV-1-specific CTL responses. This will be a dose-escalation study with increasing multiplicities of the vector.

Review--Dr. Straus (presented by Dr. Hirano)

In Dr. Straus' absence, Dr. Hirano summarized his primary review of the protocol. Most of the questions raised by Dr. Straus have been satisfactorily answered by the PIs. Dr. Straus asked about the advantage of using a retrovirus vector to express the HIV *env* gene instead of direct injection of recombinant *env* protein. The PIs responded that the *env* protein produced by the retrovirus vector will be processed within the cell to induce cellular immunity while direct injection of *env* protein will only induce a humoral immune response. Dr. Straus asked whether the CTL responses induced by the injected HIV strain would cross react with other strains of HIV virus. Dr. Straus questioned the exclusion of patients being treated by antiviral drugs since this protocol is not an efficacy study and since antiviral drugs should not alter CTL responses. He asked why the PIs propose crossing-over with the placebo recipients in this Phase I study.

Dr. Hirano agreed with Dr. Straus' concerns about the exclusion of patients receiving antiviral drug therapy, particularly in reference to the statement that azidothymidine

(AZT) (an antiviral drug) increases CTL responses in HIV patients. Dr. Hirano noted that the PIs have stated that all safety testing on the vector/producer cell line are satisfactory. This statement is meaningless unless one knows the specific fractions of the production lot that were tested and how these aliquots correlate with the proposed patient doses. Safety testing in terms of patient-dose equivalents would be a more meaningful approach. She questioned whether the phenotypes of CD8(+) and CD4(+) CTL responses differ between mice and humans.

Review--Dr. Zallen

Dr. Zallen raised specific concerns regarding the proposed protocol. Why is patient selection restricted to those individuals who have no early signs of acquired immunodeficiency syndrome (AIDS) and who have a CD4(+) count above 400? In their written responses, PIs responded that this protocol is a Phase I study in which patients are targeted in an effort to minimize any possible masking of product-attributable toxicities due to the onset of progressive disease. The Informed Consent document is very well written, and she suggested several minor changes such as using the term "gene transfer" instead of "gene therapy." There may be a possible conflict of interest in the informed consent process; namely, since the PIs are the primary care physicians, they are not the most appropriate persons to act as negotiators for obtaining informed consent. She noted that the PIs included limited research credentials in their submission. What is the function of the Monitoring Board? How does the Monitoring Board affect PIs' decision-making? How will patient confidentiality be assured?

Other Comments

Dr. Smith proposed a hypothetical event in which an HIV virus with an altered host range could result from the retrovirus transfection protocol used in these patients. Theoretically, such an event could occur if an amphotropic *env* gene from the packaging cells is transduced by the retrovirus vector into the HIV-producing cells of the patients. An altered host range of HIV then could arise either through a recombination event between HIV and the amphotropic *env* gene or through transient production of a pseudotype virus of HIV with the amphotropic envelope. A pseudotyped HIV could result in uncertain toxicity in the patient or others. Dr. Smith inquired whether this hypothetical scenario could pose a problem in this study. Dr. Geiduschek suggested that the use of another unrelated vector, such as a DNA virus, could circumvent such a scenario.

Dr. Parkman noted that there is a threshold virus dose in eliciting CTL responses in animal experiments. The dose proposed for this human study is below the threshold for CTL responses in animal models. Ms. Meyers commented that the Informed Consent document is well written and suggested that a statement should be included about a

request for autopsy. Dr. Post asked the investigators to elaborate on their statement that using a co-cultivation procedure, Viagene has detected RCR that has previously escaped detection by regular supernatant assays. He inquired whether antiviral drugs would be administered if patients' CD4(+) counts fall below 400. He noted that the formulation of the virus preparation is proprietary information; therefore, it was not submitted for RAC review. He asked why Viagene submitted this protocol for RAC review if they do not receive NIH funding.

Dr. Walters called on Dr. Wivel to explain the circumstances that led to this protocol being submitted for RAC review. Dr. Wivel explained that the *NIH Guidelines* requires RAC review only for those studies that: (1) are funded by the NIH, and (2) involve collaboration with NIH-funded investigators. Submission of this proposal is on a voluntary basis since no NIH funding is involved. As to the question of whether a vote should be taken for this protocol, Dr. Wivel answered that the standard voting procedure will be used, but the vote is not binding on the PIs if they have FDA approval to proceed with the study. Dr. Miller commented that it is an encouraging development that private companies are voluntarily submitting their human gene transfer protocols for RAC review. As to the proprietary information, RAC has previous experience with reviewing such materials in executive session.

Investigator Response--Dr. Mento

Dr. Steven Mento, Vice-President of Research and Development at Viagene, responded to the RAC's questions and comments. He explained that Viagene is currently sponsoring an ongoing protocol which was not reviewed by the RAC, which is an *ex vivo* study involving a first generation product that will be used in a limited number of clinical trials. The current protocol utilizes a direct vector product that will be used for future trials involving NIH funded institutions. Therefore, Viagene has voluntarily complied with the RAC review process. After some discussion, the RAC adopted the position that it will review this protocol with the same standard as for other protocols. It was unnecessary for the RAC to go into executive session.

Dr. Mento responded to Dr. Post's question regarding detection of RCR. The RCR safety data has been submitted to FDA as part of the master file. A co-cultivation assay using *Mus dunni* cells has been used as a sensitive method for the detection of RCR in packaging cell lines. Dr. Mento described an instance in which a producer cell line tested negative for all assays on supernatant. When the *Mus dunni* co-cultivation assay was employed, a low level of RCR contamination [1 RCR particle per 10⁷ plaque forming units (PFU)] was detected. A high standard of quality assurance is maintained for virus preparations; therefore, the producer line was discarded. Drs. Post and Miller inquired about the nature of the RCR breakout. Dr. Mento explained that the breakout appeared to be an amphotropic recombinant arising in the packaging cell line. Dr.

Mento speculated that the packaging cell line may have a block that prevents re-infection by amphotropic virus. Such a block could prevent RCR detection by other assays such as the S⁺L⁻ amplification test.

Regarding the issue of virus formulation, Dr. Mento explained that the excipient added to increase stability of retrovirus particles has previously been tested in humans. The excipient formulation is proprietary information and has not been submitted in this application. He stated that Viagene would submit the formulation on a confidential basis if necessary. Dr. Miller stated that the formulation of the excipient should not present a problem from the recombinant DNA aspect. Dr. Chase, however, was concerned approving a protocol in which information has been withheld. Dr. Parkman and Mr. Capron suggested that the formulation could be submitted to ORDA and reviewed by RAC members on a confidential basis.

Responding to Dr. Straus' question about allowing patients on the placebo group to crossover to receive the retrovirus preparations at a later time, Dr. Mento stated that this decision was made based on the fact that the placebo group was originally intended to be a control for the active treatment group in the assessment of CTL responses and other parameters. Recent studies have shown that comparisons within one group at different time points are more meaningful than comparisons to a placebo group. In the current design, the placebo group is primarily for comparing the short-term adverse effects of vector administration. Patient crossover at a later time point will not affect the investigational results.

Dr. Galpin responded to the question of excluding patients on AZT from participation in this study. Most of the patients in this study will have CD4(+) cell counts over 500; therefore, the study can be conducted without the necessity for AZT administration. On the question of confidentiality, patient identity will be known only to the physicians, not Viagene. The physicians negotiating informed consent will not be the PIs of this study.

In answering a question raised by Dr. Smith on the possibility of altering the HIV host range through the amphotropic envelope gene of the packaging cells, Dr. W. French Anderson, University of Southern California, said that such a scenario is highly unlikely. Recombination between the HIV gp120 and murine gp70 is unlikely to occur. Any recombinant would likely be less toxic than gp120 alone. If any of the HIV genome were packaged in the murine amphotropic envelope, it would be destroyed by the host complement system. Dr. Mento commented that the retrovirus backbone used in the vector does not contain the amphotropic *env* gene. The vector preparations should be safe if they are free of RCR. Dr. Miller agreed that this hypothetical risk is a highly speculative situation. Mr. G'dali Braverman from Act Up expressed his support of Viagene submitting this protocol for RAC review. It is an encouraging phenomenon that industry has voluntarily established dialogue with the RAC and the public. In closing, Dr.

Mento agreed to provide the excipient formulation on a confidential basis. Dr. Post proposed this formulation should be reviewed by Dr. Straus and Ms. Grossman.

Committee Motion

A motion was made by Dr. Miller and seconded by Dr. Secundy to approve the protocol with the following stipulations: (1) the excipient formulation of the retrovirus vector will be reviewed by Dr. Straus and Ms. Grossman, and (2) the Informed Consent document will include a request for autopsy. The motion passed by a vote of 16 in favor, 0 opposed, and 2 abstentions.

XIII. ADDITION TO APPENDIX D OF THE NIH GUIDELINES REGARDING A HUMAN GENE THERAPY PROTOCOL ENTITLED: *A MOLECULAR GENETIC INTERVENTION FOR AIDS - EFFECTS OF A TRANSDOMINANT NEGATIVE FORM OF REV/DR. NABEL*

Review--Drs. Smith

Dr. Walters called on Dr. Smith to present his primary review of the protocol submitted by Dr. Gary J. Nabel of the University of Michigan Medical Center, Ann Arbor, Michigan. Dr. Smith stated that the underlying hypothesis of this protocol is that transduction of genes that express a transdominant inhibitory HIV protein into HIV-infected and non-infected lymphocytes may reduce productive viral replication in these cells. Such action may result in prolonged survival of the transduced cells *in vivo* in patients with HIV. In the long term, it is hoped that prolonged survival could result in a beneficial clinical effect for HIV-infected patients by prolonging the latent phase of the infection. CD4(+) cells will be obtained from the peripheral blood of HIV patients, stimulated to grow *in vitro*, and transduced with a retrovirus vector carrying a transdominant inhibitory *rev* gene of HIV (Rev M10). As a control, an aliquot of CD4(+) cells from the same patient will be transduced with a similar vector that contains a frameshift mutation at the initiation codon of *rev* that prevents its expression (Δ Rev M10). Both sets of transduced cells will be reinfused in the patient and observed for differential rates of survival. The endpoints of the study are: (1) to measure survival of the two differentially transduced CD4(+) cell populations, and (2) to monitor the immune status of these patients. The PI has proposed two methods of gene transfer, transduction with a retrovirus vector carrying the *rev* mutant gene and biolistic transduction of the mutant gene in a plasmid DNA construct. The latter method would avoid many of the safety issues pertaining to retrovirus vectors, but its transduction efficiency has not yet been demonstrated.

Dr. Smith said that the original protocol contained insufficient information about the number of patients, the amount of blood necessary for transduction, and an incomplete

Informed Consent document, etc. Responses by the PI subsequently clarified most of these questions. The investigators must provide a more detailed description of the transduction procedure.

Review--Dr. Straus (presented by Dr. Smith)

In Dr. Straus' absence, Dr. Smith summarized his review of the protocol. Dr. Straus raised similar concerns to those outlined previously. He was satisfied with the responses to his questions by the PI and recommends approval of the protocol.

Review--Dr. Zallen

Dr. Zallen explained that her initial concerns were about the number of patients that will be accrued onto this study and the criteria for patient selection. Several modifications were suggested regarding the Informed Consent document. The PI responded to one of her concerns stating that 3 patients will be treated in each arm of this study with different transduction vectors. There are still several questions remaining about patient selection, and the Informed Consent language is too technical and needs simplification.

Other Comments

Ms. Meyers commented that the Informed Consent document should include the following: (1) a recommendation that contraception be used by males, (2) a section about compensation for research-related injury, (3) a description of long-term patient follow-up, and (4) a request for autopsy. Mr. Capron explained that the insurance company will cover the costs associated with standard treatment, and that the research grants will cover costs related to research. Dr. Nabel agreed.

Dr. Parkman asked two questions: (1) Is there data demonstrating that transduction of the mutant *rev* gene blocks HIV production in lymphocytes infected with HIV? and (2) Since there are 2 transduction schemes and 2 perturbations of each scheme, CD3/IL-2 and CD3/CD28 lymphocyte stimulation, are there 2 or 4 patient groups in this study? Dr. Miller stated that all 3 vector sequences were screened through GenBank, and that no open reading frames or harmful sequences were identified.

Investigator Response--Dr. Nabel

Dr. Nabel explained that the objective of this study is to introduce a protective gene into the uninfected CD4(+) lymphocytes of HIV patients to prevent HIV replication when later infected with the virus. *Rev* is one of the essential HIV genes required for the transition from latent to active infection. The function of the *rev* gene product is to

facilitate the transport of HIV RNA from the nucleus to the cytoplasm for completion of the virus replication cycle. The transdominant mutant form of the *rev* protein inhibits this transport and keeps HIV in the latent phase. Therefore, the ultimate goal is to protect the CD4(+) cells that are not yet infected with HIV.

In addressing the safety issues raised by Dr. Smith, Dr. Nabel stated that *in vitro* transduction by the retrovirus vector will be performed in the presence of anti-HIV drugs and *Pseudomonas* exotoxin, which kills cells that have HIV gp120 *env* protein. Such measures will minimize the generation of HIV from these cells. Dr. Nabel stated that the probability of generating a novel strain of HIV by the amphotropic vector is extremely small as discussed in Drs. Galpin and Casciato's protocol. Regarding the trial design, Dr. Nabel said that a total of 12 patients will be enrolled, 4 groups of 3 patients each. There will be 2 transduction methods; in each transduction group, there will be 2 subgroups with different lymphocyte stimulation procedures, CD3/IL-2 and CD3/CD28.

Dr. Nabel agreed to simplify the language in the Informed Consent document in order to make it more understandable to patients. Patients will not be required to pay for any costs associated with the gene transfer aspects of the protocol. Ms. Meyers mentioned that long-term follow-up of patients is important if any unforeseeable event occurred in the gene transfer trial.

Responding to Dr. Miller's question, Dr. Nabel said that in cell culture experiments, the Rev M10 mutant protects lymphocytes from producing HIV by 3 to 4 orders of magnitude. However, this protection is not absolute; it can be overwhelmed by higher titer of virus production. The proposed human study is designed to test *in vivo* protection. No animal model is currently available in which to test this hypothesis. Dr. Miller asked whether the present proposal is different from the protocol submitted by Dr. Clay Smith, Memorial Sloan Kettering Cancer Center, New York, New York, which was previously deferred by the RAC. Dr. Post mentioned that in Dr. Smith's protocol, all experiments were performed in cell lines. The present protocol has additional data that was obtained using fresh peripheral blood lymphocytes from HIV patients.

Responding to Dr. Zallen's question about the long-term planning of the present study, Dr. Nabel said future directions depend on the outcome of this initial trial. If no protective effect is observed, useful knowledge will still be obtained in terms of transducing CD4(+) cells. But if there is positive protective effect, these transduced CD4(+) cells can be isolated and expanded in tissue culture and reinfused back to patients for a more aggressive therapeutic trial. Dr. Parkman remarked that in this initial stage of the study, the word "benefit" in the Informed Consent document is not appropriate. Dr. Smith stated that he was still concerned about the safety issues surrounding the use of an amphotropic retrovirus vector in HIV patients but felt that he could defer to those members with greater expertise in this area. He stated that he

would abstain from voting on this protocol. Dr. Post noted his intention to abstain from voting on this protocol due to a conflict of interest.

Committee Motion

A motion was made by Dr. Parkman and seconded by Dr. Miller to approve the protocol contingent on the submission of a revised Informed Consent document including the following: (1) a recommendation that contraception be used by males as well as females, (2) a request for autopsy in the event of death, and (3) an explanation of long-term patient follow-up. The motion passed by a vote of 14 in favor, 0 opposed and 4 abstentions.

XIV. CLOSED SESSION FOR EXPEDITED REVIEW OF A HUMAN GENE TRANSFER PROTOCOL

A closed session of the full RAC was held to provide an in-depth review of the May 11, 1993, expedited approval by the NIH Director of a human gene transfer protocol submitted by Drs. Ivor Royston and Robert Sobol of the San Diego Regional Cancer Center, San Diego, California. Approval was granted for additional treatments using a new retrovirus vector, G1NaCvi2. The RAC concurred with the recommendations of the intramural and extramural reviewers and the NIH Director's decision to approve the expedited review protocol.

In open session the following day, Dr. Walters summarized the committee motion on Drs. Sobol and Royston's protocol and read a statement prepared by the RAC regarding expedited review procedures.

"The RAC recommends that ORDA urge Principal Investigators to provide timely notice when considering a minor modification to a previously approved protocol or a request for expedited review. This recommendation is intended to facilitate patient care and the quality of the review process."

XV. AMENDMENT TO SECTION IV-C-3-c OF THE NIH GUIDELINES REGARDING PUBLICATION OF THE RECOMBINANT DNA TECHNICAL BULLETIN AND DATA MANAGEMENT OF THE NIH-APPROVED HUMAN GENE TRANSFER PROTOCOLS/MS. WILSON

Ms. Wilson proposed to discontinue publication of the *Recombinant DNA Technical Bulletin (Bulletin)* on the basis that most of the information is duplicated elsewhere. RAC meeting minutes are currently published in the *Journal of Human Gene Therapy* (Mary Ann Liebert, Inc.). *Federal Register* notices, including proposed and major actions to the *NIH Guidelines*, are distributed through ORDA to IBC Chairs, RAC members,

and other interested parties. Ms. Wilson proposed an amendment to the *NIH Guidelines* that would replace the requirement for publication of the *Bulletin* with a statement that ORDA will serve as a focal point for data management of NIH-approved human gene transfer protocols. The *NIH Guidelines* Section IV-C-3-c currently reads:

"IV-C-3. *The Office of Recombinant DNA Activities.* ORDA shall be responsible for the following...

"IV-C-3-c. Publishing the *Recombinant DNA Technical Bulletin*..."

Section IV-C-3-c will be amended to read:

"IV-C-3. *The Office of Recombinant DNA Activities.* ...ORDA shall be responsible for the following...

"IV-C-3-c. Serve as the focal point for data management of NIH-approved human gene transfer protocols as required in the Reporting Requirements section of the *"Points to Consider."*

Committee Motion

A motion was made by Dr. Post and seconded by Dr. Secundy to accept the proposed amendment. The motion passed by a vote of 16 in favor, 0 opposed and 2 abstentions.

XVI. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* REGARDING *RICKETTSIA PROWAZEKI* -- TRANSFER OF A CHLORAMPHENICOL RESISTANCE MARKER TO AN AVIRULENT STRAIN/DR. POLICASTRO

Review--Dr. Krogstad

Dr. Walters called on Dr. Krogstad to present his primary review of a request submitted by Dr. Paul Policastro of the NIH, Hamilton, Montana, to introduce a chloramphenicol-resistant marker into an attenuated strain of *R. prowazeki* (Madrid E). Dr. Krogstad stated that *R. prowazeki* is a human pathogen that causes epidemic typhus. Since chloramphenicol and tetracycline are the antibiotics used in treating human infections, transfer of the chloramphenicol resistant gene in this organism is a cause for concern.

The PI has provided little information on the genetics of the avirulent strain of *Rickettsia* to be used in this study or the rationale for choosing chloramphenicol as opposed to other less critically useful antibiotics. Dr. Krogstad recommended that the RAC defer approval of this request.

Review--Dr. Post

Dr. Post stated that he originally considered this request to be reasonable since the PI proposed the introduction of an antibiotic resistant gene in an attenuated strain of *Rickettsia*. Since Dr. Krogstad (an infectious disease expert) has raised many serious concerns about this experiment, Dr. Post recommended that the RAC defer approval of this proposal.

Other Comments

Dr. Haselkorn noted that an outside expert, Dr. David O. Wood of Department of Microbiology and Immunology, University of South Alabama, Mobile, Alabama, submitted a review of this request. Dr. Wood states that introduction of an antibiotic resistance gene into a Class 3 pathogen poses major concerns. Dr. Haselkorn questioned whether the PI has explored the use of other antibiotics that would be useful in cell culture but that are not critical for the treatment of human infection.

Committee Motion

A motion was made by Dr. Post and seconded by Dr. Straus to defer approval of the request. The motion to defer approval passed by a vote of 20 in favor, 0 opposed and no abstentions. The request was deferred until the investigator submits the following data for full RAC review: (1) data demonstrating that the construct is safe and useful, and (2) *in vitro* data demonstrating the selective advantage of chloramphenicol resistance over other selectable markers.

XVII. AMENDMENT TO SECTION III-A-4 OF THE *POINTS TO CONSIDER REGARDING THE TERM SUBJECTS/DR. PARKMAN*

Dr. Parkman proposed an amendment to the *NIH Guidelines* regarding the use of the term "subjects". To close a potential loophole that could conceivably allow investigators to bypass RAC review for single patient protocols, he proposed that the current language should be revised to cover single or multiple patient protocols.

Section III-A-4 currently reads:

"III-A-4. Deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into *human subjects* [21]..."

Throughout the *Points to Consider* document the term "subject" and "subjects" are used.

Section III-A-4 will be amended to read:

"III-A-4. Deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into *one or more human subjects* [21]..."

The term "subject" and "subjects" will be changed throughout the *Points to Consider* and the *NIH Guidelines* to "one or more human subjects."

Committee Motion

A motion was made by Dr. Motulsky and seconded by Ms. Meyers to accept the proposed amendment. The motion passed by a vote of 20 in favor, 0 opposed, and no abstentions.

XVIII. DISCUSSION REGARDING INITIATION OF NIH-APPROVED HUMAN GENE TRANSFER PROTOCOLS AT SATELLITE INSTITUTIONS

The RAC initiated discussion regarding the possible format that would be employed for the review and approval of human gene transfer protocols conducted at satellite institutions. The RAC noted several issues that would have to be examined in further detail, i.e., uniform Informed Consent documents, quality control, and the cell transduction process, such as *in vivo* versus *ex vivo*, if transduction is performed by the sponsor or at a satellite site, etc. The RAC recommended that this issue should be examined in further detail and discussed as a future agenda item.

XIX. ADDITION TO APPENDIX D OF THE NIH GUIDELINES REGARDING A HUMAN GENE THERAPY PROTOCOL ENTITLED: *GENE THERAPY FOR THE TREATMENT OF RECURRENT PEDIATRIC MALIGNANT ASTROCYTOMAS WITH IN VIVO TUMOR TRANSDUCTION WITH THE HERPES SIMPLEX THYMIDINE KINASE GENE*/DRS. RAFFEL AND CULVER

Review--Dr. Hirano

Dr. Walters called on Dr. Hirano to present her primary review of the protocol submitted by Dr. Corey Raffel of the Childrens Hospital of Los Angeles, Los Angeles, California, and by Dr. Kenneth Culver of Iowa Methodist Medical Center, Des Moines, Iowa. This protocol is very similar to two protocols by Drs. Oldfield and Culver previously approved by the RAC. The approach is to use the murine vector producer cell line (PA317) to deliver the *Herpes simplex* thymidine kinase (HS-tk) by means of a retrovirus vector. Tumor cells that express the HS-tk gene will be killed by administration of the antiviral drug, ganciclovir. The major difference between this protocol and the other two previously approved protocols is that the proposed study will involve children, 2-18 years of age, with malignant brain tumors. Dr. Hirano asked the PIs to clarify several questions: (1) What are the statistics on treatment responses?

Since this protocol is a Phase II study addressing the question of treatment efficacy, it is important to clearly state the definition of responses and criteria of efficacy. It is unclear how the "stop/proceed" criterion was derived. A total of 15 patients will be placed on this study initially. If no patients respond to treatment, then the trial will be closed. If at least 1 patient responds, then an additional 14 patients will be enrolled in this study. How were these numbers derived statistically? What are the re-treatment criteria? Patients who demonstrate minimal, partial, or complete responses to therapy will be considered for re-treatment with 3 cycles of virus producer cells (VPC). What is considered a response, and what are the rationale for these criteria? If these questions are adequately addressed by the PIs, the protocol should be approved.

Review--Dr. Geiduschek

As a note on safety, the PI has indicated that 8 brain tumor patients have now been treated, and no evidence of toxicity has been observed. Dr. Geiduschek asked the PIs to confirm whether this statement is true. Most of the other points raised by Dr. Geiduschek concerned the wording in the Informed Consent document, such as explicitly stating that the cells to be given to patients are *mouse* cells. Dr. Geiduschek expressed his satisfaction to responses from the PIs on his remarks.

Other Comments

Dr. Zallen asked why there was no assent form included for children participating in this trial. Dr. Raffel stated that at the Childrens Hospital of Los Angeles the same form is used for consent by parents and assent by children, a practice adopted by the Committee for Clinical Investigation. Dr. Parkman, the past Chair of the Committee for Clinical Investigation, agreed with Dr. Raffel's statement. Dr. Zallen questioned whether a 10 year old is capable of understanding the consent form prepared for adults. Dr. Motulsky shared the same sentiments. Mr. Capron expressed the need to prepare a form understandable to minors. Since different institutions have their own rules for preparing these documents, Ms. Meyers proposed uniform federal regulation. Dr. Walters stated that proposing such regulation is beyond the mandate of the RAC, but a letter could be drafted addressing this issue for institutions funded by NIH.

Dr. Doi inquired whether the young animals responded similarly to adults in the animal studies. Is there additional information about the toxicity issue? Ms. Wilson noted that a report had been filed with ORDA regarding a possible adverse reaction on the Oldfield protocol. One patient demonstrated asymptomatic gliosis following treatment. Dr. Miller said that the vector used in this trial is different from the vector used in the previous protocols. Does this vector have the same activity as the vector used for the animal experiments? He asked if the PIs are planning to treat a large number of patients in this study before obtaining results from previous protocols.

Investigators' Responses--Drs. Raffel and Culver

Dr. Raffel explained the rationale of proposing this protocol for the treatment of pediatric brain tumors. The biology of astrocytoma in children and adults is different, e.g., childhood tumors do not possess p53 tumor suppressor gene mutations. Responding to Dr. Hirano's question on the "stop/proceed" criterion, Dr. Raffel said that tumor recurrence occurs quickly without additional therapy. A failure will be considered tumor recurrence within 3 months of vector producing cell (VPC) administration. Patient with recurrent tumors will not be eligible for retreatment. Otherwise, additional rounds of 3 treatment cycles will continue. Regarding the issue of an assent form for children, Dr. Raffel agreed to make changes as permitted by his IRB. As to the animal studies, results were obtained in young rats, which may not be comparable to 2 year old humans. This study excludes patients below 2 years of age because their brain development is incomplete.

Responding to a question by Dr. Miller about differences between the proposed vector and the vector used in the 2 previously approved human trials, Dr. Culver stated that the vector and the VPC proposed for this study are the same vector used for other studies. Several vectors were compared in animal studies. Regarding the toxicity issue of the two ongoing brain tumor trials, Dr. Culver stated that no acute toxicity was encountered, but a chronic or subacute adverse reaction was reported by Dr. Oldfield. Dr. Culver stated that in this protocol, attempts will be made to select patients with rapidly growing tumors and to remove as much necrosis as possible from the tumor mass to allow for optimal "bystander" effects of the VPC on surrounding tumor cells.

In response to Dr. Hirano's question about the statistics of the stopping rule, Dr. Raffel said that statistical analysis indicates that if 15 patients are treated and no responses are observed, the chance of missing an effective treatment is less than 5%. This outcome is the cut off point. If a response is observed, then a total of 30 patients will be treated.

A lengthy discussion ensued regarding the necessity for obtaining proper assent from children. The suggestion was made that there are children's assent forms for other protocols that could be used as an example, e.g., the assent form for the ADA protocol.

Committee Motion

A motion was made by Dr. Hirano and seconded by Dr. Geiduschek to approve the protocol. An amendment was made by Dr. Zallen and seconded by Ms. Meyers that the investigators will submit an assent document that will inform children of the experimental procedures and associated risks. This document must be reviewed and approved by Drs. Zallen and Secundy. The motion to approve the amendment passed by a vote of 19 in favor, 0 opposed, and 2 abstentions. The motion to approve the protocol

passed by a vote of 19 in favor, 0 opposed, and 2 abstentions.

XX. ADDITION TO APPENDIX D OF THE NIH GUIDELINES REGARDING A HUMAN GENE THERAPY PROTOCOL ENTITLED: *USE OF A RETROVIRAL VECTOR TO STUDY THE TRAFFICKING PATTERNS OF PURIFIED OVARIAN TIL POPULATIONS USED IN INTRAPERITONEAL ADOPTIVE IMMUNOTHERAPY OF OVARIAN CANCER PATIENTS - A PILOT STUDY/DR. FREEDMAN*

Review--Dr. DeLeon

Dr. Walters called on Dr. DeLeon to present her primary review of the protocol submitted by Dr. Ralph S. Freedman of the University of Texas MD Anderson Cancer Center, Houston, Texas. Dr. DeLeon stated that this pilot study proposes the use of gene marking to monitor the trafficking patterns of purified tumor infiltrating lymphocytes (TIL) that are employed in the treatment of patients with epithelial ovarian carcinoma. The first objective of this study is to determine whether purified CD3(+)/CD8(+) ovarian TIL, which have been expanded in IL-2, can be transduced with the G1Na retrovirus vector carrying the neomycin resistant (neo^R) gene. Secondly, the PI will determine the distribution and survival of the transduced cells following intraperitoneal injection. The PI plans to study a total of 20 patients. TIL will be obtained from tumor specimens at the time of surgery, expanded in tissue culture, and returned to patients. At 1, 2, and 3 months post-TIL administration, a group of 6 patients will undergo surgical biopsy at the site of tumor and surrounding normal tissue to monitor for neo^R by PCR. The proportion of CD8(+) and CD4(+) cells will be monitored by fluorescence activated cell sorter analysis. She stated that this proposal is a straightforward gene marking protocol and presents no major concerns since it is similar to other previously approved protocols. Dr. DeLeon posed two minor issues: (1) omission of the required written report of adverse reactions to ORDA in the *Points to Consider*, and (2) the requirement that patients must bear the cost of the investigational agents and procedures. The PI agreed that this requirement is inappropriate, and that this section of the Informed Consent document will be amended.

Review--Dr. Chase

Dr. Chase stated that the protocol is poorly written; there is no clear description of the structure of the experiment. Dr. Chase expressed great doubt about the validity of the proposed statistical considerations of the experimental results. He raised five major concerns: (1) The treatment plan is not uniform. (2) Standard oncologic treatment is indistinguishable from the experimental procedures. (3) The Informed Consent document is unclear and requires patients to bear some of the experimental costs. (4) The IBC placed a contingency on approval of the protocol, yet the PI has stated that the contingency has been met, and (5) The discussion of the expected outcome is different

from the research hypothesis.

Review--Dr. Secundy

Dr. Secundy stated that the investigators had adequately responded to her concerns regarding the time frame. However, questions about the exclusion and inclusion criteria remain.

Other Comments

Dr. Parkman stressed that the risks associated with the administration of gene marked cells, which will have no therapeutic effect, should be weighed against the importance of the data that will be obtained. Dr. Geiduschek stated the critical criterion for approval of this protocol is the consensus that this study will yield knowledge that will benefit the treatment of future patients. Dr. Chase has indicated that this protocol will not yield scientifically beneficial information as it is currently constructed. Therefore, Dr. Geiduschek stated that he is inclined to defer approval of the proposal. Dr. Parkman asked the PI to elaborate on how selectivity will be demonstrated, i.e., what are the methods that will be employed to demonstrate preferential trafficking of TIL to tumor versus adjacent normal tissues? Dr. Carmen said that the gene transfer procedure is not adequately described in the Informed Consent document in language that will be easily understood by lay persons.

Investigator Response--Dr. Freedman

Dr. Freedman stated that this protocol is an extension of an ongoing study of adoptive immunotherapy for the treatment of ovarian cancer. TIL will be marked with the neo^R gene to determine the trafficking pattern of transduced cells. Ovarian cancer primarily involves the peritoneal surface and serosa. The objective of this study is to determine whether TIL preferentially localize to tumors. Marked TIL will be monitored by quantitative PCR. He presented preliminary tissue culture data.

Dr. Parkman asked about the percentage of TIL that are transduced *in vitro*. Dr. Freedman responded that technical difficulties have been encountered in transducing CD8(+) cells, and no definitive result has been obtained. Dr. Miller commented that if transduction frequency is low, marked TIL will be difficult to track in tumor and surrounding normal tissues. Dr. Parkman said that if the sensitivity of detecting marked cells by PCR is unknown, the selectivity of TIL trafficking to tumor sites cannot be determined. Dr. Freedman responded that TIL will be administered intraperitoneally close to the tumor sites; therefore, a large fraction of TIL should localize to tumor. Dr. Freedman said that Dr. Deisseroth's laboratory will perform the PCR analysis. Dr. Deisseroth presented PCR data. Dr. Miller stressed the technical difficulty of

performing quantitative PCR analysis and stated that adequate control experiments have not been included. Dr. Post said that this proposal has many shortcomings.

Committee Motion

A motion was made by Dr. Dronamraju and seconded by Dr. Parkman to defer approval of the protocol based on the following concerns: (1) data demonstrating efficient transduction of TIL, (2) insufficient information regarding demonstration of selectivity, i.e., specific trafficking of TIL to tumor, (3) incomplete statistical analysis, (4) the Informed Consent document must be revised in simplified language, and (5) concerns about patient responsibility for research-related costs must be addressed. The motion to defer approval of the protocol passed by a vote of 18 in favor, 0 opposed, and no abstentions.

XXI. ADDITION TO APPENDIX D OF THE NIH GUIDELINES REGARDING A HUMAN GENE THERAPY PROTOCOL ENTITLED: *IMMUNIZATION OF MALIGNANT MELANOMA PATIENTS WITH INTERLEUKIN-2-SECRETING MELANOMA CELLS EXPRESSING DEFINED ALLOGENEIC HISTOCOMPATIBILITY ANTIGENS*/DRS. DAS GUPTA, COHEN AND RICHARDS

Review--Dr. Smith

Dr. Walters called on Dr. Smith to present his primary review of the protocol submitted by Drs. Tapas K. Das Gupta and Edward P. Cohen of the University of Illinois College of Medicine, Chicago, Illinois, and Dr. Jon M. Richards of the University of Chicago, Chicago, Illinois. Dr. Smith stated that this protocol is a Phase I study of 12 patients with advanced stage melanoma. Patients will be injected with a melanoma cell line (Mel-4) that has been transduced with a gene encoding IL-2. These transduced cells will act as an immunogenic vaccine. The hypothesis is that IL-2 secreting allogeneic melanoma cells will induce B and T cell anti-tumor responses. The endpoint for this study is toxicity; however, minor endpoints will include measurements of the induction of antibodies against HLA and melanoma associated antigen, and where possible, induction of CTL-mediated responses and other parameters. The transduced Mel-4 cells will be irradiated with 10,000 rads. He stated his concern that the murine data was generated using viable melanoma cell immunization; however, the human study proposes using irradiated cells that could impair IL-2 secretion. Is IL-2 production in Mel-4 cells stable following irradiation? What are the levels of IL-2 production in human cell lines? Dr. Smith stated that he was originally concerned that the eligibility criteria in this protocol were too stringent, but the PI has relaxed the criteria. The Informed Consent document implies that the purpose of this trial is therapeutic, yet requires patients to pay for part of the research associated costs. This issue must be addressed by the PIs. In summary, additional information regarding the background data, particularly the transduced cell

line to be used for the human study, should be provided prior to approval of this protocol.

Review--Dr. Dronamraju

Dr. Dronamraju noted that the patient population is divided into several categories according to their ethnic background and gender. What is the scientific rationale for excluding certain ethnic groups?

Other Comments

Mr. Capron stated that the Informed Consent document does not include a recommendation for male/female contraception, a description of the financial responsibility of patients, or a request for autopsy. Is this study a gene therapy or gene marking protocol? Dr. Walters commented that this immunization study has therapeutic intent. Ms. Meyers objected to the use of the word "vaccine" in the Informed Consent document and suggested several other minor changes.

Dr. Geiduschek said that the murine data do not directly correlate with the human proposal, e.g., different cell numbers and treatment regimen. Dr. Post asked the PI to clarify an earlier statement about RCR assays. Dr. Parkman said that critical data were not submitted prior to the meeting; therefore, it is impossible for the RAC to evaluate the protocol.

Investigator Response--Dr. Cohen

Dr. Cohen presented *in vivo* murine data demonstrating prolonged survival of mice that were immunized with IL-2 secreting allogeneic mouse fibroblasts expressing melanoma associated antigens. Dr. Parkman remarked that the murine experiments were performed with non-irradiated cells. Dr. Cohen agreed that the cells were not irradiated, and that all tumors eventually recurred. Responding to Dr. Smith's question about the effect of irradiation, Dr. Cohen presented *in vivo* murine data demonstrating increased survival with irradiated IL-2 cells over control animals; however, survival was less than with non-irradiated cells. Dr. Smith noted that the cells used for the animal experiments were irradiated with 5,000 rads not 10,000 rads as proposed for the human study. Experiments have not been submitted using IL-2 producing cells that have been irradiated with 10,000 rads. Responding to Dr. Post's question about RCR assays, Dr. Cohen said that these safety assays will be performed by Microbiological Associates, Inc., Rockville, Maryland

Dr. Smith expressed his concern at the lack of *in vitro* human data. He said that he is inclined to recommend deferral of this protocol until additional data is submitted. Data

should demonstrate whether IL-2 is produced at 24, 48, or 72 hours following irradiation with 10,000 rads. Dr. Miller suggested that data be submitted demonstrating that 5,000 rads inactivates the growth of human cells and that these cells continue to secrete IL-2. Dr. Post recommended that RCR co-cultivation assays should be submitted using irradiated human cells. Dr. Miller added that a positive control experiment demonstrating RCR in human melanoma cells will aid in the interpretation of RCR assays on these cells. Dr. Miller said that he had screened the vector sequence through GenBank, and that no harmful sequences or open reading frames were identified.

Committee Motion

A motion was made by Dr. Smith and seconded by Ms. Grossman to defer approval of the protocol until the investigators return to the RAC with the following: (1) data demonstrating the efficiency of transduction in Mel-4 cells; (2) data demonstrating viability, IL-2 production, and *in vivo* murine effect of irradiated transduced cells (either 5,000 or 10,000 rads); (3) rationale for ethnic eligibility criteria; (4) complete responses to the *Points to Consider*; and (5) RCR testing data demonstrating safety of the vector preparation. The motion to defer approval of the protocol passed by a vote of 19 in favor, 0 opposed, and 1 abstention.

XXII. ADDITION OF APPENDIX D OF THE NIH GUIDELINES REGARDING A HUMAN GENE THERAPY PROTOCOL ENTITLED: *HUMAN MDR GENE TRANSFER IN PATIENTS WITH ADVANCED CANCER/DRS. HESDORFFER AND ANTMAN*

Review--Dr. Parkman

Dr. Walters called on Dr. Parkman to present his primary review of the protocol submitted by Drs. Charles Hesdorffer and Karen Antman of Columbia University College of Physicians and Surgeons, New York, New York. Dr. Parkman stated that this protocol is a resubmission of a proposal previously submitted by Dr. Arthur Bank at the March 1993 RAC meeting. The objective of this protocol is to evaluate expression of MDR-1 in the progeny of CD34(+) bone marrow stem cells in recipients of autologous bone marrow (ABM) transplants for the treatment of advanced cancer. Patients will be followed after ABM transplantation to evaluate MDR-1 expression. At the time the original protocol was submitted, the RAC was concerned about clinical aspects of the protocol. The original protocol was approved for the non-invasive treatment of melanoma. Ovarian, brain, and breast cancer are the proposed targets of this study. The PIs have submitted additional data demonstrating the lack of RCR using co-cultivation with *Mus dunni*. The PIs have removed all aspects concerning the post-transplant administration of Taxol from the protocol. The revised protocol focuses on the transduction of CD34(+) cells rather than "human hematopoietic stem cells." The Informed Consent document has been revised. Since Taxol administration is no longer

part of the gene therapy protocol, which will be performed as part of another Phase I/II study, the RAC must discuss how the issue of toxicity related to Taxol administration will be considered.

Review--Dr. Krogstad

Dr. Krogstad stated that one major concern is the possibility of inadvertent transduction of malignant cells in the marrow. Sensitive techniques detect bone marrow metastases in 30 to 50% patients of advanced breast cancer. Originally, he suggested that a "spiking" experiment be performed to simulate the inadvertent transduction of tumor cells in bone marrow. If tumor cells are not detected following isolation of CD34(+) bone marrow cells that have been deliberately added to the initial sample, the risk of inadvertent transduction of tumor cells is minimal. The PI has performed such experiments, and the results are encouraging. The CD34(+) cell selection procedure efficiently removes contaminating tumor cells from bone marrow. The Informed Consent document should be revised to avoid the suggestion that the present procedure is "safe", and should suggest that contraception should be used by males and females. The revised protocol is substantially improved over the previous submission.

Review--Mr. Capron

Mr. Capron stated that the question of whether Taxol will be involved in the present study is somewhat ambiguous in the writing of the protocol and the consent form. The headings and some technical terms of the Informed Consent document should be revised in simplified language.

Other Comments

Dr. Miller expressed concern about the RCR testing data that was submitted. The PIs state that an extremely safe packaging cell line will be used that is incapable of producing wild-type retroviruses; and therefore, does not pose any public health hazard. The seriousness of RCR testing has been greatly overlooked. The reverse transcriptase assay is not as sensitive as other retrovirus rescue assays for the detection of RCR that have been approved by the RAC. In this protocol, a large volume of retrovirus supernatant will be used to infect bone marrow cells; however, RCR assays were performed with 1 ml aliquots. Dr. Miller stated that these assays are unacceptable. Dr. Miller said that the Informed Consent document does not clearly state whether the study involves Taxol administration. He agreed with Dr. Parkman's concern about ambiguities in the scope of this study.

Ms. Meyers said that she had the following concerns about the Informed Consent document: (1) the statement that patients are responsible for costs associated with side

effects due to the treatment, (2) there should be a statement about long-term follow-up, (3) a section about patient confidentiality, and (4) a request for autopsy.

Investigator Response--Dr. Hesdorffer

In response to questions about Taxol administration in this protocol, Dr. Hesdorffer explained Taxol is an issue separate from the transduction protocol. The purpose of this study is to introduce MDR-1 into CD34(+) cells and to evaluate long-term expression following ABM transplantation. If patients relapse or have residual disease after ABM transplantation, they or their physicians can elect to enter the Taxol protocol in which Taxol will be administered by a dose escalation regime. The question of enhancement of MDR-1 expression by Taxol will be assessed in the subsequent protocol. Dr. Hesdorffer agreed to revise the Informed Consent document to indicate that Taxol treatment is not part of the initial gene transfer experiment.

Regarding Dr. Krogstad's question about metastatic disease involving bone marrow, Dr. Hesdorffer said ovarian and brain tumors rarely metastasize to the bone marrow. For breast cancer, the PI will select patients without metastatic disease. Data suggests that the monoclonal antibody selection procedure eliminates the majority of tumor cells still present in the bone marrow. Since most patients who participate in this study have advanced cancer with no other alternative therapies, the additional risk is of minimal concern. Dr. Hesdorffer said that the Informed Consent document will be revised according to Mr. Capron and Ms. Meyers' suggestions. With regard to financial responsibility for research-related injuries, this unresolved issue was discussed when the protocol was presented previously. Responding to Dr. Miller's questions about RCR assays, Dr. Hesdorffer said that *Mus dunni* co-cultivation and S+L- assays have been performed in addition to the reverse transcriptase assays. Twenty percent of the clinical grade supernatant will be assayed for RCR prior to use in patients. Dr. Miller said that the safety assays described by Dr. Hesdorffer were not included in this submission.

Dr. Miller said that data have not been submitted demonstrating the transduction of human bone marrow cells. Dr. Hesdorffer said that these data were included in the original submission. Drs. Parkman and Geiduschek said that the RAC should recommend approval of this protocol contingent on the submission of additional data. Ms. Grossman expressed her interest in reviewing the additional data.

Committee Motion

A motion was made by Dr. Parkman and seconded by Dr. Krogstad to approve the protocol. Approval of the protocol is contingent on the review and approval of the following: (1) data demonstrating the transduction efficiency of human CD34(+) cells, and (2) a description of assays that will be performed on the clinical grade supernatant.

The motion passed by a vote of 11 in favor, 5 opposed, and 3 abstentions.

XXIII. ADDITION TO APPENDIX D OF THE NIH GUIDELINES REGARDING A HUMAN GENE THERAPY PROTOCOL ENTITLED: *GENE THERAPY FOR HUMAN BRAIN TUMORS USING EPISOME-BASED ANTISENSE cDNA TRANSCRIPTION OF INSULIN-LIKE GROWTH FACTOR/DR. ILAN*

Review--Dr. Miller

Dr. Walters called on Dr. Miller to present his primary review of the protocol submitted by Dr. Joseph Ilan of Case Western Reserve University School of Medicine and University Hospitals of Cleveland, Cleveland, Ohio. Dr. Miller explained that an Epstein-Barr virus (EBV) vector will be used that directs the synthesis of antisense insulin growth factor (IGF)-1 RNA and inhibits IGF-1 synthesis in glioblastoma cells obtained from patients with incurable brain tumors. Modified cells will be lethally irradiated and injected subcutaneously to stimulate immune destruction of tumor at peripheral sites. *In vivo* studies in rats support the feasibility of this technique. An episomal plasmid-based vector, which encodes EBV nuclear antigen-1 and antisense IGF-1, will be introduced into tumor cells by liposome transfection. The cells will be lethally irradiated prior to injection; therefore, there are no vector-related safety issues. The animal data supports the potential clinical utility of this approach. He asked if the investigators have additional data demonstrating the transduction of the antisense construct and inhibition of IGF-1 in human cells. Antisense IGF-1 expression is driven by a metallothionein promoter, which is inducible by metal ions. He asked whether the level of metal ions in the patients' body is sufficient to induce the promoter to express antisense RNA. Dr. Miller recommended that the protocol should be approved.

Review--Dr. Geiduschek

Dr. Geiduschek explained that the episomal vector used in this study is a circular piece of DNA that replicates within the cell nucleus; therefore, it is not an integral component of the chromosomes. Dr. Miller added that the reason for using an episomal vector for antisense expression is that it will replicate to high copy number when transfected into target cells and will produce high levels of antisense RNA necessary to inhibit IGF-1 production.

Dr. Geiduschek said that this protocol proposes a potential therapy of an otherwise incurable disease in a conceptually coherent and generally persuasive way. The majority of his initial concerns have been responded to with the following exceptions: (1) Does the PI have experience generating the required quantities of cells from surgically acquired specimens? and (2) Does the PI have experience in producing large quantities

of antisense expressing cells for clinical use?

Review--Ms. Meyers

Ms. Meyers said that the section of the Informed Consent document that explains "sense" and "antisense" gene expression may not be understandable to lay persons. She suggested that the term "cures" should be changed to "treatments."

Other Comments

Dr. Parkman asked if the PI will select patients with gliomas that over-express IGF-1. What is the definition of over-expression? Does over-expression in tumor cells established *in vitro* correlate with primary tumors *in vivo*? What is the transduction efficiency in human cells using liposome transfection? Does the rate of transduction correlate with therapeutic responses observed in animal studies?

Dr. Post said that this proposal is very interesting; however, important human data is missing. Dr. Ilan responded that he had indicated in his written responses that the human data would be provided at the RAC meeting. Dr. Post stated that it is not an acceptable practice to allow PIs to withhold critical data until the meeting.

Investigator Response--Dr. Ilan

Dr. Ilan presented animal data that supported the basis for the proposed human trial. Rat C6 glioma cells express IGF-1 and form rapidly growing tumors in syngeneic animals. These cells lose tumorigenicity when transfected with the antisense IGF-1 cDNA vector. Subcutaneous injection of transfected C6 cells into rats prevented tumor formation at the injection site and at distal sites. These anti-tumor effects result from a glioma-specific immune response involving CD8(+) lymphocytes. Antisense blocking of IGF-1 expression may reverse a phenotype of the tumors that allows C6 glioma cells to evade the immune system. Modified C6 cells act as a vaccine against C6-induced tumors. Dr. Ilan presented additional data on other tumor types, such as osteosarcoma and rat teratocarcinoma.

Drs. Miller and Geiduschek stated that although the animal data is encouraging, little information is known about the transduction of human cells. In response to Dr. Geiduschek's concern about the length of time required to grow a sufficient quantity of cells, Dr. Ilan explained that patients will receive radiation treatment for 2 months following surgery. During this time, tumor cells will be established in culture, transduced, and expanded to the necessary number of cells required for implantation. Dr. Ilan said that he is capable of reproducibly generating sufficient quantities of cells for treatment. Dr. Parkman inquired about the percentage of cells that are transduced.

Dr. Ilan responded that the cells will be selected in hygromycin; therefore, the remaining cells will be 100% transduced. Dr. Miller asked about the IGF-1 efficiency of inhibition in these transfected human cells. Dr. Ilan answered that IGF-1 production is completely inhibited; however, he stated that he did not have data demonstrating this inhibition. Dr. Miller stated that he had requested these data several weeks before the meeting. Critical data have not been submitted for review. Drs. Miller and Geiduschek stated that they could only recommend approval of this protocol contingent on the submission and review of IGF-1 inhibition data.

A lengthy discussion ensued about the inappropriateness of investigators withholding critical data prior to a RAC meeting. The RAC members said that this practice is an ongoing procedural problem that must be addressed. Dr. Parkman suggested that data not included in the written proposal should not be allowed to be presented at the RAC meeting. Dr. Krogstad said that the *Points to Consider* states that written responses from the PI are due to ORDA 2 weeks before the meeting. Dr. Parkman requested that the *Points to Consider* should be amended to prevent the submission of data immediately prior (< 2 weeks before meeting) and during the committee meeting.

Committee Motion

A motion was made by Dr. Miller and seconded by Dr. DeLeon to approve the protocol contingent on the submission of the following: (1) data demonstrating inhibition of IGF-1 expression by the antisense construct in human tumor cells, (2) data demonstrating repeated success in establishing primary cultures from fresh human tumors, and (3) data demonstrating efficiency of the transduction procedure. This document must be reviewed and approved by Drs. Straus, Post, Miller, Geiduschek, and Ms. Grossman. The motion to approve the protocol passed by a vote of 19 in favor, 0 opposed, and no abstentions.

XXIV. ADDITION TO APPENDIX D OF THE NIH GUIDELINES REGARDING A SEMLIKI FOREST VIRUS (SFV) VECTOR EXPRESSION SYSTEM--REDUCTION OF PHYSICAL CONTAINMENT FROM BL3 to BL2/DR. TEMPLE

Review--Dr. Krogstad

Dr. Walters called on Dr. Krogstad to present his primary review of the proposal submitted by Dr. Gary F. Temple of Life Technologies, Inc., Gaithersburg, Maryland. This request is a resubmission that was previously reviewed at the September 1992 RAC meeting. The investigators are requesting a reduction in the physical containment level from Biosafety Level (BL) 3 to BL2 for their Semliki Forest virus (SFV) cloning vector. Dr. Krogstad stated that this reclassification would make Life Technologies' SFV cloning kit more widely available and marketable. Since there is no jurisdiction in this area by

FDA or any other federal agency, the RAC is the only review body for this particular proposal. Dr. Krogstad provided a clinical overview of SFV infection. In Tübingen, Germany, 1978, a laboratory worker death was reported in association with SFV infection. Although the cause of death was not clearly established, the containment classification of SFV was raised from BL2 to BL3. In 1990, there was an outbreak of SFV infection among a group of French soldiers in Africa. Clinical manifestations included fever and other mild symptoms of systemic infection. Other instances of infection in laboratory workers have been reported who demonstrated seroconversion and developed antibodies against SFV. Dr. Krogstad stated that the RAC must exercise extreme caution in their consideration of this proposal since a judgement must be rendered without adequate clinical information.

Review--Dr. Miller

Dr. Miller outlined the issues that remained at the time this proposal was last reviewed by the RAC. Little information was known about the frequency of recombination yielding replication-competent SFV under conditions for using the cloning vector system. In addition, the incidence of seropositivity of laboratory workers exposed to this virus had not been determined. On this resubmission, the applicants have determined the frequency of helper virus production in the system. The results indicate that helper virus will be readily detectable. Two strategies were employed to reduce the possibility of generating helper virus. One involves the separation of helper function on different RNA molecules. The frequency of generating helper virus is 10^{-3} per vector infectious unit. The other strategy involves mutation of the spike protease region to prevent virus activation, and the maximum rate of helper production with this mutation is 2×10^{-4} . These rates are much higher than the 10^{-6} that was originally proposed by the PIs. These results indicate the real potential for generating SFV helper virus in the gene expression vector product. Regarding the risk to laboratory workers from using this system, the risks are very real and must be considered. Seroconversion rates are still too low to evaluate the potential for disease following infection. The RAC must consider that a fatal infection was previously reported. Given that one can expect helper virus production at some rate, the risk to laboratory workers cannot be ignored.

Dr. Miller said that he recommends reclassification of this cloning vector from BL3 to BL2 provided that potential customers are adequately informed of the potential risks associated with the system. The investigators must provide customers with: (1) an information sheet that describes the potential health risks, (2) appropriate methods to be used for virus inactivation, (3) a simple helper virus assay to detect replication competent SFV, (4) a description of the symptoms that would be expected in the event of SFV infection, and (5) a warning indicating the potential for SFV recombination.

Dr. Miller said that the investigators' response to the RAC's initial concerns appeared to

downplay the potential pathogenicity of this virus. Two *ad hoc* reviewers have also submitted written critiques of the proposal. The *ad hoc* reviewers, Dr. Alan L. Schmaljohn of U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick at Frederick, Maryland, and Dr. Dennis W. Trent of National Center for Infectious Diseases, Fort Collins, Colorado, recommended use of this system at BL2 containment, however, they also emphasized the necessity to exercise caution.

Ms. Meyers inquired whether SFV is infectious to animals. Dr. Miller responded that it is possible that SFV can be transmitted by mosquitos from humans to animals, causing the virus to become endemic in the region.

Review--Dr. Post

Dr. Post stated that there is no valid scientific reason for classifying SFV and Sindbis virus in different containment categories. It appears that the classification of SFV at the BL3 containment level resulted from a single fatal case report. This case involved a subject suffering from chronic bronchitis and a strain of SFV that was unnaturally passaged through animals. Considering the long-term safe laboratory use with SFV and the epidemiological data about the natural infection of humans, SFV should not be considered a particularly virulent agent. The vector proposed by Life Technologies, Inc., is a disabled version of the wild-type virus. He said that he would vote to approve the request to reclassify this cloning vector system for use at BL2 provided that the applicants supply proper instructions for the safe handling of this vector cloning system.

Other Comments

Dr. Parkman asked whether this virus can be easily inactivated. Dr. Straus responded that it is easily inactivated and should not be an issue; however, he has serious concern about the general use of this virus by nonvirologists who are inexperienced at handling viral agents. The history of the safe use of SFV at BL2 in the past involved virologists experienced with use of these viruses. Dr. Straus was concerned that when the cloning vector is marketed, it will be used by laboratory personnel who do not have proper virology training.

Investigator Response--Dr. Temple

In response to Dr. Krogstad's question of seroconversion, Dr. Temple said that in Dr. Robert E. Shope's laboratory at Yale University, 2 out of 15 laboratory workers demonstrated borderline conversion; and in Dr. L. Kaariainen's laboratory at the University of Helsinki in Finland, 8 out of 16 laboratory workers were seropositive for SFV. These two laboratories all performed their experiments at BL2 containment. Regarding Dr. Miller's interpretation of the recombination frequency, Dr. Temple said

that it is his belief that there is a low level of replication competent SFV that could emerge from large volumes of cells and cloning vectors in the laboratory setting.

Dr. Temple stressed that the cloning vector is derived from an attenuated strain of SFV. Symptoms of SFV infection are very similar to infection by Sindbis virus. SFV is spread from animal to animal by mosquitos; human infection is incidental. The proposal specifically states that the vector is not going to be used in animal experiments. Dr. Temple said that passages of SFV through animals generally increase its virulence, as occurred in the one reported fatality.

Dr. Miller emphasized that the investigators failed to include an information sheet describing safe usage and potential risk as was requested.

Committee Motion

A motion was made by Dr. Miller and seconded by Dr. Motulsky to defer approval of the proposal. The proposal was deferred until the investigators return to the RAC with the following: (1) a product information sheet informing customers of the potential health risk of the expression system, standard methods to be used for virus inactivation, a helper virus assay to detect SFV, and a description of symptoms and procedures to be followed in the event that SFV infection occurs in a laboratory worker (including methods to prevent transfer to insect vectors and environmental spread); and (2) SFV inactivation data. The motion to defer the request passed by a vote of 16 in favor, 2 opposed, and 1 abstention.

XXV. ADDITION TO APPENDIX D OF THE NIH GUIDELINES REGARDING THE POXVIRUS VECTORS NYVAC, ALVAC, AND TROVAC--REDUCTION OF PHYSICAL CONTAINMENT FROM BL2 TO BL1/DR. PAOLETTI

Review--Dr. Gay

Dr. Walters called on Dr. Gay, Chief Staff Veterinarian of the Office of Veterinary Biologics, U.S. Department of Agriculture (USDA), to present his *ad hoc* review of the proposal submitted by Ms. Arvilla L. Trag (on behalf of Dr. Enzo Paoletti) of Virogenetics Corporation, Troy, New York. Dr. Gay explained that this proposal requests a reduction in physical containment for 3 recombinant pox vectors NYVAC, ALVAC, and TROVAC from BL2 to BL1. Dr. Gay said that the Office of Veterinary Biologics is responsible for licensing all veterinary biologics in the United States. License applications have previously been reviewed for Rhone Merieux, Inc., for 2 recombinant vector vaccines that were constructed with poxvirus-based vectors, TROVAC and ALVAC. TROVAC-NDV, a Newcastle Disease-Fowlpox Vaccine is based on TROVAC. ALVAC-RG, a Rabies Vaccine is based on ALVAC. The USDA

Office of Veterinary Biologics has conducted risk analyses and has recommended that the physical containment levels for TROVAC and ALVAC be reduced from BL2 to BL1.

Review--Dr. Moss (Presented by Dr. Walters)

In the absence of Dr. Moss, NIH, Bethesda, Maryland, an *ad hoc* expert on poxviruses, Dr. Walters summarized Dr. Moss' written review of the request. Dr. Moss is of the opinion that there is little or no inherent risk to individuals in working with these viruses. The only possible risk that could be foreseen would be inadvertent immunization with a recombinant protein expressed by these vectors. Dr. Moss recommended the use of the proposed vectors at BL1 containment unless there is reason to believe that immunizing amounts of the gene products would provide an additional risk.

Other Comments

Mr. Capron asked about risk of inadvertent immunization alluded to in Dr. Moss' review. Dr. Straus described one example in which an individual exposed to HIV proteins produced by poxvirus vectors could be seropositive for HIV even though the individual is not infected by HIV. Another example would be respiratory syncytial virus infection in which a vaccinated individual may react more severely to viral infection than an unvaccinated child. Such risks are not due to the vectors *per se* but are due to the particular proteins expressed by these vectors.

Many other written comments had been submitted in support of the proposed reclassification based on the highly attenuated characteristics and restricted host ranges of these vectors. These written reviews were by the following scientists: Dr. W. K. Joklik of Duke University Medical Center; Dr. Peter W. Mason of the Agricultural Research Service, USDA; Dr. Joanne Maki, Rhone Merieux, Inc.; Dr. David E. Lanar, Walter Reed Army Medical Center; Dr. Bert Jacob of Arizona State University; Dr. Robert E. Shope of Yale University; and Dr. Mary Lou Clements of Johns Hopkins School of Hygiene and Public Health.

Investigator Response--Dr. Paoletti

Dr. Paoletti explained that he is requesting reclassification of three vectors that are currently classified as Class 2 pathogens. NYVAC is based on the Copenhagen strain of vaccinia virus in which 18 viral genes have been deleted; ALVAC is based on an attenuated strain of canarypox; and TROVAC is based on an attenuated strain of fowlpox virus. Dr. Post asked what additional advantages would be gained from this reclassification since permission for field testing has already been granted by the USDA. Dr. Paoletti said that approval of this request would facilitate the transfer and

production of viral materials and allow for the inexpensive disposal of vaccinated animals. If these vectors are reclassified for use at BL1, there would be substantial commercial benefit. Dr. Post suggested one possible problem associated with the use of NYVAC (the vaccinia-derived vector) in patients with eczema. Such a vector may cause disseminated disease in these individuals. Dr. Paoletti said that in immune compromised animal models, no evidence of disseminated infection has been observed. The vector will probably be administered by subcutaneous or intramuscular routes as opposed to dermal scarification for the parental vaccinia virus.

Committee Motion

A motion was made by Dr. Straus and seconded by Dr. Carmen to approve the request. The motion was passed by a vote of 15 in favor, 0 opposed, and 1 abstention.

XXVI. REPORT FROM THE WORKING GROUP ON EXEMPT REVIEW OF HUMAN GENE TRANSFER PROTOCOLS/DR. PARKMAN

Dr. Parkman submitted a draft document entitled: *Cover Sheet for Exempt Review of Human Gene Marking Protocols*. The RAC submitted several changes to the proposed document which might be used for the decentralized review of human gene transfer protocols that are identical to studies previously reviewed and approved by the RAC. The working group will continue to refine the draft document for discussion at the next RAC meeting.

XXVII. A DRAFT LETTER TO THE NIH DIRECTOR REGARDING COMPENSATION FOR RESEARCH-RELATED INJURIES

Dr. Walters resumed discussion on a letter circulated earlier by Dr. Parkman regarding the issue of compensation for research-related injuries. This letter would be forwarded to the NIH Director as a follow-up to the January 6 letter.

Dr. Parkman suggested that compensation be incorporated in the forthcoming President's health care reform proposal. The following statement was approved by the RAC as follows:

"The sense of the Recombinant DNA Advisory Committee (RAC) is that the present discussions about national health care reform provide an opportunity for the NIH Director to address the issues relating to providing health care for patients who are injured as a result of participating in clinical research approved by NIH-mandated IRBs. A report of the President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research, *Compensating for Research Injuries* (1982), demonstrated that other nations which

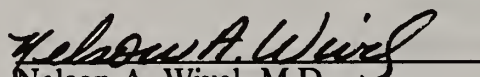
provide health care coverage for all citizens avoid the problems that arise in attempting to design a separate compensation system for patients injured because of their participation in medical research. Thus, the RAC supports the inclusion in basic universal health care of coverage for injury received through participation in approved clinical research. It is the strong desire of the RAC that the NIH Director would call this issue to the attention of the individuals formulating the President's health care reform proposal."

Committee Motion

A motion was made by Dr. Carmen and seconded by Ms. Meyers to approve the letter. The motion passed by a vote of 14 in favor, 0 opposed, and no abstentions. This follow-up letter will be forwarded to the NIH Director by Dr. Walters. [Executive Secretary's Note: Dr. Walters, Chair of the RAC, forwarded the follow-up letter to Dr. Healy on June 28.]

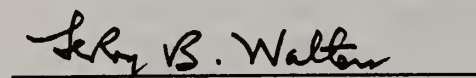
XXVIII. ADJOURNMENT

Dr. Walters adjourned the meeting at 4:45 p.m. on June 8, 1993.


Nelson A. Wivel, M.D.
Executive Secretary

I hereby acknowledge that, to the best of my knowledge, the foregoing Minutes and Attachment are accurate and complete.

Date: 9 / 9 / 93


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**RECOMBINANT DNA ADVISORY COMMITTEE (RAC)
DATA MANAGEMENT REPORT - JUNE 1993**

Protocol #	PI(s)	Institution	RAC Approval Date	NIH Approval Date	M/T	Patient Population	Date First Patient Treated	Vector/ Gene	Packaging Cell	Vector Supplier	Maximum Patient Accrual	Patient Accrual Date	In Vivo Evidence of Gene Transfer (Human)	Problems with Gene Transfer Procedure	Adverse Effects	Number of Patient Deaths	Protocol Status (Open/Closed)
8810-001*	Rosenberg	NIH	10-3-88	3-2-89	M	Cancer	5-22-89	LNL6/Neo ^R	PA317	GTI	Unlimited	10	Yes	No	No	8	Open
9007-002*	Blaese	NIH	7-31-90	9-6-90	T	SCID due to ADA Deficiency	9-14-90	LASN-ADA	PA317	GTI	10	2	Yes	No	No	0	Open
			2-10-92 (Amend)	4-22-92 (Amend)			5-13-93	G1NaSvAd	PA317	GTI							
9007-003	Rosenberg	NIH	7-31-90	9-6-90	T	Cancer	1-29-91	TNF-Neo ^R (pLT12SN)	PA317	GTI/Chiron	50	10	Yes	No	No	7	Open
9102-004*	Brenner, et. al.	St. Jude Children's	2-4-91	7-12-91	M	AML	9-9-91	LNL6/Neo ^R	PA317	GTI	12	12	Yes	No	No	3	Closed
9105-005*	Brenner, et. al.	St. Jude Children's	5-31-91	7-12-91	M	NBL	1-27-92	LNL6/Neo ^R	PA317	GTI	10	6	Yes	No	No	0	Open
9105-006*	Brenner, et. al.	St. Jude Children's	5-31-91	7-12-91	M	NBL	1-16-92	LNL6/Neo ^R	PA317	GTI	10	3	P	No	No	1	Open
9105-007*	Deisseroth	MD Anderson	5-31-91	7-12-91	M	CML	7-31-92	LNL6/Neo ^R	PA317	GTI	10	8	Yes	No	ND-1 failure to engraft	3	Open
9105-008	Ledley, et. al.	Baylor College of Medicine	5-30-91	7-12-91	M	Acute Hepatic Failure	P	LNL6/Neo ^R	PA317	GTI	6	0	NA	NA	NA	NA	Open
9105-009*	Lotze	Univ. of Pittsburgh	5-30-91	1-17-92	M	Melanoma	3-3-92	LNL6/Neo ^R	PA317	GTI	20	2	No	No	No	1	Open
9110-010*	Rosenberg	NIH	10-7-91	10-15-91	T	Cancer	10-8-91	TNF-Neo ^R (pLT12SN)	PA317	GTI/Chiron	5	3	Yes - 2 patients	No	No	2	Open
9110-011	Rosenberg	NIH	10-7-91	10-15-91	T	Cancer	3-12-92	GiNaCVi2 (IL-2)	PA317	GTI	5	2	Yes - 1 patient	No	No	1	Open
9110-012*	Wilson	Univ. of PA. Med. Center	10-8-91	11-14-91	T	FH	6-5-92	CMV-BA-(h)LDLR	Ψ-crip	Univ. of PA. Med. Center	5	3	Yes - 1 patient	No	No	0	Open
9202-013*	Nabel	Univ. of Michigan	2-10-92	4-17-92	T	Cancer	6-4-92	Cationic liposomes/HLA-B7 cDNA	NA	Univ. of Michigan	12	6	No	No	No	0	Open

9202-014	Cometta	Indiana University	2-11-92	4-17-92	M	AML ALL	5-15-92	GI1Na/Neo ^R	PA317	GTI	10 AML 10 ALL	3	No	No	No	1 AML	Open
9202-015	Economou	Univ. of Calif., LA	2-11-92	4-17-92	M	Melanoma Renal Cell Cancer	2-19-93	LNL6/Neo ^R and GI1Na/Neo ^R	PA317	GTI	20 Melanoma 20 Renal Cell	1 Melanoma	No	No	No	No	Open
9202-016	Freeman	Univ. of Rochester	2-10-92	2-5-93	T	Ovarian Cancer	P	HSV-TK	PA317	Univ. of Rochester	20	0	NA	NA	NA	Open	
9202-017	Greenberg Riddell	Univ. of Washington	2-11-92	4-17-92	M	HIV(+)	2-26-93	HyTK	PA317	TGC	15	1	P	No	No	0	Open
9206-018	Brenner, et. al.	St. Jude Children's	6-1-92	8-14-92	T	NBL	12-9-92	GI1NaCvi2	PA317	GTI	12	2	No	No	No	1	Open
9206-019	Oldfield	NIH	6-1-92	8-14-92	T	Brain Tumors	12-7-92	GITKSvNa	PA317	GTI	10-Primary Tumors 10-Brain Metastases	6-Primary Tumors 2-Brain Metastases	No	No	ND-1 asympt. gliosis	0	Open
9206-020*	Deisseroth	MD Anderson	6-2-92	8-14-93	M	Leukemia	P	GI1Na/Neo ^R and LNL6/Neo ^R	PA317	GTI	10	0	NA	NA	NA	NA	Open
9206-021	Gansbacher Houghton Livingston	Memorial Sloan-Kettering	6-2-92	8-14-92	T	Metastatic Melanoma	2-17-93	NAPAD/IL2	GP + env Am12	Memorial Sloan Kettering	12	3	No	No	No	0	Open
9206-022*	Gansbacher Motzer Houghton Bander	Memorial Sloan-Kettering	6-2-92	8-14-92	T	Renal Cell Cancer	3-17-93	NAPAD/IL2	GP + env Am12	Memorial Sloan Kettering	12	2	No	No	No	No	Open
9206-023	Dunbar	NIH	6-2-92	8-14-92	M	Multiple Myeloma	3-18-93	LNL6/Neo ^R and GI1Na/Neo ^R	PA317	GTI	12	2	P	No	No	0	Open
9206-024	Dunbar	NIH	6-2-92	8-14-92	M	Breast Cancer	12-7-92	LNL6/Neo ^R and GI1Na/Neo ^R	PA317	GTI	12	2	P	No	No	0	Open
9206-025	Dunbar	NIH	6-2-92	8-14-92	M	CML	P	LNL6/Neo ^R and GI1Na/Neo ^R	PA317	GTI	12	0	NA	NA	NA	NA	Open
9206-026	Walker	NIH	9-14-92	P	M	HIV +/ Identical Twin	P	LNL6/Neo ^R and GI1Na/Neo ^R	PA317	GTI	12	0	NA	NA	NA	NA	P

9209-027	Schuening	Fred Hutchinson	9-14-92	2-5-93	M	Breast Carcinoma and Hodgkin's Disease	P	LN/IL3	PA317	TGC	20	0	NA	NA	NA	NA	Open
9209-028	Schuening	Fred Hutchinson	9-14-92	2-5-93	M	Lymphoid Malign.	P	LN/G-CSF	PA317	TGC	20	0	NA	NA	NA	NA	Open
9209-029	Schuening	Fred Hutchinson	9-14-92	2-5-93	M	Identical Twins/Engraftment	P	LN/G-CSF	PA317	TGC	20	0	NA	NA	NA	NA	Open
9209-030	Deisseroth	MD Anderson	9-14-92	P	M	CLL	P	LNL6/Neo ^R and G1Na/Neo ^R	PA317	GTI	10	0	NA	NA	NA	NA	P
9209-031	Roth	MD Anderson	9-15-92	P	T	NSCLC	P	LNSX-AS-K-ras and LNSX-p53	GP + env Am12	GTI	14	0	NA	NA	NA	NA	P
9209-032	Brenner	St. Jude Children's	9-15-92	2-5-93	M	NBL	P	LNL6/Neo ^R and G1Na/Neo ^R	PA317	GTI	12	0	NA	NA	NA	NA	Open
9209-033	Loize Rubin	Univ. of Pittsburgh	9-15-92	2-5-93	T	Cancer	P	G1IL4SvNa (IL4)	PA317	GTI	20	0	P	P	P	P	Open
TOTAL					13T 20M							92 Patients					

TABLE LEGEND:

•	= Minor Modification
M	= Gene Marking Protocol
NA	= Not Applicable
ND	= Not Determined
P	= Pending
T	= Gene Therapy Protocol

[606]

ACRONYMS:

ADA	= Adenosine Deaminase
ALL	= Acute Lymphocytic Leukemia
AML	= Acute Myelogenous Leukemia
CML	= Chronic Myelogenous Leukemia
FH	= Familial Hypercholesterolemia
G-CSF	= Granulocyte - Colony Stimulating Factor
GTI	= Genetic Therapy, Inc.
HIV	= Human Immunodeficiency Virus
NBL	= Neuroblastoma
NSCLC	= Non-Small Cell Lung Cancer
SCID	= Severe Combined Immunodeficiency
TGC	= Targeted Genetics Corporation

MINOR MODIFICATIONS TO NIH-APPROVED HUMAN GENE TRANSFER PROTOCOLS JUNE 7-8, 1993, RAC MEETING

DATE OF MINOR MODIFI- CATION	DATE OF NIH APPROVAL	PROTOCOL #	PRINCIPAL INVESTIGATOR(S)	PROTOCOL TITLE	MINOR MODIFICATION
3/30/90	3/2/89	8810-001	Rosenberg, Steven A.	The Treatment of Patients with Advanced Cancer Using Cyclophosphamide, Interleukin-2, and Tumor Infiltrating Lymphocytes	Lift the cap on the number of patients accrued onto the protocol.
11/91	7/12/91	9102-004	Brenner, Malcolm K., et. al.	Autologous Bone Marrow Transplant for Children with Acute Myelogenous Leukemia in First Complete Remission: Use of Marker Genes to Investigate the Biology of Marrow Reconstitution and the Mechanism of Relapse	Use the retroviral vector, G1Na, instead of LNL6.
11/91	7/12/91	9102-005	Brenner, Malcolm K., et. al.	A Phase I/II Trial of High Dose Carboplatin and Etoposide with Autologous Marrow Support for Treatment of Stage D Neuroblastoma in First Remission: Use of Marker Genes to Investigate the Biology of Marrow Reconstitution and the Mechanism of Relapse	Use the retroviral vector, G1Na, instead of LNL6.
11/91	7/12/91	9102-006	Brenner, Malcolm K., et. al.	A Phase II Trial of High-Dose Carboplatin and Etoposide with Autologous Marrow Support for Treatment of Relapse/Refractory Neuroblastoma Without Apparent Bone Marrow Involvement	Use the retroviral vector, G1Na, instead of LNL6.
7/9/92	10/15/91	9110-010	Rosenberg, Steven A.	Immunization of Cancer Patients Using Autologous Cancer Cells Modified by Insertion of the Gene for Tumor Necrosis Factor (TNF)	Extend patient eligibility criteria to include patients with advanced breast cancer.
11/30/92	1/17/92	9105-009	Lotze, Michael T.	The Administration of Interleukin-2, ~~Interleukin-4~~ and Tumor Infiltrating Lymphocytes to Patients with Melanoma	Eliminate systemic IL-4 therapy.
12/3/92	11/14/91	9110-012	Wilson, James M.	Ex Vivo Gene Therapy of Familial Hypercholesterolemia	Increase patient accrual from 3 to 5 patients.

1/93	4/17/92	9202-013	Nabel, Gary J.	Immunotherapy of Malignancy by In Vivo Gene Transfer into Tumors	(1) addition of assays for anti-DNA antibodies on day 28 and week 8; (2) repeat chest and abdominal CTs, when indicated, at 6-8 weeks following initial treatment; (3) additional biopsies performed on days 16 and 20 and as needed to verify gene expression; (4) reduce the duration of the protocol from 16 to 8 weeks; and (5) expand patient eligibility to include those patients who have tolerated previous treatments without side effects at higher doses.
1/22/93	4/17/92	9202-013	Nabel, Gary J.	Immunotherapy of Malignancy by In Vivo Gene Transfer into Tumors	Deliver HLA-B7 DNA liposome complexes by catheter to pulmonary nodules.
3/31/93	8/14/92	9206-022	Gansbacher, Bernd, et. al.	Immunization with Interleukin-2 Secreting Allogeneic HLA-A2 Matched Renal Cell Carcinoma Cells in Patients with Advanced Renal Cell Carcinoma	Use only irradiated SK-RC-39 cell line, not SK-RC-28.
4/19/93	7/12/91	9102-007	Deisseroth, Albert B.	Autologous Bone Marrow Transplantation for Chronic Myelogenous Leukemia in which Retroviral Markers are Used to Discriminate between Relapse which Arises from Systemic Disease Remaining after Preparative Therapy Versus Relapse due to Residual Leukemic Cells in Autologous Marrow: A Pilot Trial	New transduction protocol which allows the use of autologous stroma and growth factors.
4/19/93	8/14/92	9206-020	Deisseroth, Albert B.	Use of Two Retroviral Markers to Test Relative Contribution of Marrow and Peripheral Blood Autologous Cells to Recovery After Preparative Therapy	New transduction protocol which includes the use of autologous stroma and growth factors.
4/21/93	9/6/90 Amend. 4/22/92	9007-002	Blaese, R. Michael	Treatment of Severe Combined Immune Deficiency (SCID) due to Adenosine Deaminase (ADA) Deficiency with CD34(+) Selected Autologous Hematopoietic Stem Cells	Treat newborns with ADA deficiency with ADA gene-corrected autologous hematopoietic stem cells obtained from the patients' umbilical cord and placenta.
5/17/93	4/16/93	9212-034	Crystal, Ronald G.	A Phase I Study, in Cystic Fibrosis Patients, of the Safety, Toxicity, and Biological Efficacy of a Single Administration of a Replication Deficient. Recombinant Adenovirus Carrying the cDNA of the Normal Human Cystic Fibrosis Transmembrane Conductance Regulator Gene in the Lung.	Decrease the "baseline" and "vehicle control" portions of the protocol from 1 month to 1 week each.

HUMAN GENE TRANSFER PROTOCOL

9306-043 Seigler, Hilliard F., Duke University Medical Center, Durham, North Carolina; *A Phase I Trial of Human Gamma Interferon-Transduced Autologous Tumor Cells in Patients With Disseminated Malignant Melanoma.*

Date of RAC Approval: 6/7/1993

Date of NIH Approval: 9/3/1993

Scientific Abstract

We plan to use retroviral vectors to transfer the gamma interferon (γ -IFN) gene into cancer cells from melanoma patients. It is hoped that the resultant expression of γ -IFN from the tumor cells will dramatically improve antigenic presentation by increasing the level of Class I major histocompatibility complex (MHC) proteins that present antigen to the immune system. Additionally, subsequent tumor-specific cellular immune activation may be improved by supplying this versatile cytokine, γ -IFN, to immune cells that are essential to combat human cancer.

Short-term tumor cell lines from human melanoma biopsy material will be established. The cell culture will then be transduced with the γ -IFN retroviral vector. After selection for transduced cells by growth in the antibiotic, G418, the cells will be tested for sterility, lethally irradiated so that they can no longer divide, and the γ -IFN-expressing autologous tumor cells will be re-injected into the same patient. The study will determine safety, clinical response (tumor burden), and biological response (immune responses). The resultant increase in specific immunity against the now highly immunogenic gene-modified tumor cells may result in a significant response against the endogenous, unmodified metastatic tumors.

APPENDIX B

Nontechnical description of protocol

We plan to deliver genetic information ("DNA") to the tumor cells of melanoma patients. Because it is difficult to deliver DNA directly to cells, we plan to use replication defective viruses ("vectors") that efficiently deliver the DNA to living cells. These vectors cause the patients tumor cells to produce an important protein called gamma interferon (γ -IFN). γ -IFN is an important protein because it causes the tumor cell to be brought to the attention of the patient's immune system. It is hoped that the resultant expression of γ -IFN from the tumor cells will dramatically improve immune responses that combat human cancer.

Tumor cells from human melanomas removed surgically from the patient will be grown in the laboratory under sterile conditions. The cell culture will then be placed in contact with the vector that contains the DNA coding for γ -IFN. After a process which removes tumor cells which have not taken up the γ -IFN DNA, the cells will be tested for sterility, irradiated with X-rays so that they can no longer grow, and the tumor cells expressing γ -IFN will be re-injected into the same patient. The study will determine if the approach is safe, if the patient's clinical condition is improved, and whether immune responses against the tumor have been improved.

HUMAN GENE THERAPY PROTOCOL

A PHASE I TRIAL OF HUMAN GAMMA INTERFERON- TRANSDUCED AUTOLOGOUS TUMOR CELLS IN PATIENTS WITH DISSEMINATED MALIGNANT MELANOMA

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1.0 OBJECTIVES

We plan to use retroviral vectors to transfer the gamma interferon (γ -IFN) gene into cancer cells from melanoma patients. It is hoped that the resultant expression of γ -IFN from the tumor cells will dramatically improve antigenic presentation by increasing the level of Class I major histocompatibility complex (MHC) proteins that present antigen to the immune system. Additionally, subsequent tumor-specific cellular immune activation may also be improved by supplying this versatile lymphokine, γ -IFN, crucial to the activation of immune cells that, in turn, are essential to combat human cancer.

Short-term tumor cell lines from human melanoma biopsy material will be established. The cell cultures will then be transduced with the γ -IFN retroviral vector. After selection for transduced cells by growth in the antibiotic, G418, the cells will be tested for sterility, lethally irradiated so that they can no longer divide, and the γ -IFN-expressing autologous tumor cells will be injected back into the tumor biopsy donor. The study will determine safety, clinical response (tumor burden), and biological response (immune responses). The resultant increase in specific immunity against the now highly immunogenic gene-modified tumor cells may also result in a significant response against the endogenous, unmodified metastatic tumors.

2.0 INTRODUCTION AND RATIONALE

2.1 CANCER IMMUNOLOGY

Host immunity is an important mechanism by which malignant tumors can be kept in check or even cleared. Established tumors are therefore under selective pressure to develop means of evading the immune system. Although the mechanisms by which tumors evade the immune system are undoubtedly varied and complex, at least one mechanism appears to derive from the fact that the levels of Class I MHC proteins are unusually low on most tumors (1, 2). Since Class I MHC proteins are essential for the presentation of intracellular antigen to the immune system (3, 4), suboptimal levels of MHC proteins may prevent effective presentation of tumor-specific antigens. The immune system is therefore relatively blind to the tumor cell, at least with regard to MHC Class I-restricted cytotoxic T lymphocytes (CTL). A second mechanism by which tumors could evade the immune system is to generate suppressor T cells or other immune depressing substances to prevent the normal activation of potent anti-tumor immunity (immune "anergy") (5-7). An effective immunotherapy must necessarily overcome one or both of these phenomena.

Methods for improving the immune responses against tumors, especially CTL responses, are likely to be therapeutically beneficial to cancer patients. A variety of approaches have been used to engender non-specific augmentation of the immune systems of cancer patients. For instance, lymphokines such as IL-2, α -IFN, and more recently, γ -IFN have proven to be therapeutically useful, although efficacy is limited and toxicity associated with systemic administration is considerable (8-10). γ -IFN and IL-2 presumably function as growth and activation factors (11-15) to tumor-reactive immune cells such as CTL, macrophages, natural killer (NK), and lymphokine activated killer / tumor infiltrating lymphocytes (LAK/TIL) (16) at the site of the tumor, possibly to overcome immune suppression. γ -IFN also increases the expression of MHC proteins on the surface of tumor cells to improve antigenic presentation (17, 18). The remaining systemically-available lymphokine protein presumably does not contribute to tumor-specific immunity, but does lead to significant toxicity. Systemic injection of lymphokines is thus a highly inefficient way to deliver these critically important proteins to their site of action, the tumor.

Another approach which has been used extensively by ourselves and others to activate the immune system of cancer patients is active, specific immunotherapy (ASI). In this approach, a patient's own tumor is removed and dissociated to a single cell suspension that is inactivated and re-injected, with or without additional immunological adjuvants (19-23). It is thought that tumor-specific antigens are somehow presented more effectively to the immune system when the tumor is presented in this manner. The resultant activation of cytolytic immune cells (such as CTL) have had modest but statistically significant response rates (19, 20).

Another possibility is to activate anti-tumor immunity by combining the tumor specificity of ASI with the general immune stimulation induced by lymphokines, such as γ -IFN. One approach that would assure the continued presence of γ -IFN locally around tumor cells is to introduce constitutively expressed lymphokine genes into the tumor cells. The resultant constitutive, low level production of γ -IFN should specifically increase the level of MHC (the antigen presenting proteins) on the surface of tumor cells. In addition, γ -IFN should be secreted locally around the tumor and thus aid the growth and activation of local immune cells (such as CTL, macrophages, and NK cells, 16) as they respond specifically to tumor antigen. The concentrated, local presence of the immune-activating γ -IFN may thus help overcome immune anergy. The total amount of lymphokine should then be quite low, but it should be concentrated where it would be most effective, namely at the site of tumor cells. The overall effect of lymphokine gene transfer would be to "flag" the tumor cell as something that the immune system should carefully scrutinize and respond against. The resultant boost in tumor specific immunity could result in improved responses compared with non-gene-modified ASI approaches (Fig 1).

We (see section 3.3) and others (24-30) have demonstrated the potential utility of lymphokine gene delivery in general, and γ -IFN gene delivery specifically (28-30), in murine tumor model systems. The data have been sufficiently encouraging to warrant clinical investigation in cancer patients.

2.2 RETROVIRAL VECTOR PACKAGING CELL LINES

Retroviruses are positive-strand RNA viruses that pass through a double-stranded DNA stage after infection (31,32). Viral DNA integrates into the host genome to form a provirus, which contains the viral genes encompassed on either end by viral long terminal repeat (LTR) elements which are required for integration. After integration, viral genes are expressed from the provirus by utilizing host cell machinery and transcriptional promoter and termination *cis* elements found within the viral LTRs. The full length viral RNA transcript (the next generation viral genome) is recognized via a specific "packaging signal" (Ψ) found in the viral RNA genome and encapsidated into an infectious viral particle by the viral proteins. The retroviral life cycle is generally non-lytic for the cell, and allows continuous viral production over a long period of time.

The retroviral vector used in these studies is based upon Moloney murine leukemia virus (MoMLV), that was engineered so that its extraneous viral coding sequences have been replaced with a desired gene (32-34), such as γ -IFN. Unlike true viruses, the recombinant vector is incapable of replication since its genome lacks all of the viral structural genes. Any gene of interest can replace the protein coding region of the viral genome and become packaged within a packaging cell line (PCL) into virion particles for transduction, provided that it is contiguous with the essential *cis* elements (i.e., Ψ , LTRs, and sequences required for priming of DNA synthesis) of the genome.

The viral proteins needed for encapsidation and vector production must then be supplied in a PCL. The PCL is a cell line which expresses the MoMLV structural proteins, and as a result produces empty virion particles which lack the viral genome required for replication. Viral provector DNA introduced into the PCL will act as a template for the production of the RNA vector genome, which is then packaged into the previously empty virions, generating retroviral vector particles (33,34).

For clinical studies, the most important safety concern with the use of retroviral vectors is the inherent propensity of murine retroviral PCLs to generate replication-competent retrovirus (RCRV) after introduction of a vector (35). This can theoretically occur by homologous recombination between the retroviral provector DNA and either 1) the DNA encoding the MoMLV structural genes present in the PCL (36), or 2) the endogenous proviruses found universally in murine cells (37), the species from which all the MLV-based PCLs previously used for human gene therapy

studies were generated. In fact, even murine cell lines lacking vector can be induced to produce xenotropic RCRV (38) which can replicate in human cells.

Another safety concern with murine PCLs used for retroviral vector production is that endogenous defective proviruses in the murine genome are expressed (e.g., VL30 sequences), recognized by the retroviral structural gene products of murine PCLs, and delivered and expressed in target cells with an efficiency at least comparable to that of the desired vector (39). The delivery of these undesirable retroviral-like sequences and the possibilities for generating replication-competent retrovirus suggest that murine cell lines may be less than ideal for the production of murine retroviral vectors for human therapeutics.

Due to the safety risks associated with the generation of RCRV by homologous recombination with endogenous sequences, we have developed MLV-based retroviral PCLs from a canine cell line, D17. The MLV structural genes ("gag/pol" and "env") are also genetically unlinked, to decrease the chance of retrovirus generated by homologous recombinations between the therapeutic provector DNA and the DNA encoding the MoMLV structural genes present in the PCL. These changes should help alleviate many of the safety concerns described above.

2.3 CLINICAL APPROACH

We propose to utilize retroviral vectors to deliver and express the γ -IFN gene to human tumors in order to improve ASI for human malignancies. It is hoped that this approach will improve antigenic presentation and subsequent tumor-specific immune activation in cancer patients by creating an optimal microenvironment around the tumor cells for the activation of cell mediated immune responses. Short-term tumor cell cultures will be established from human tumor biopsy material. The resultant cell cultures will be transduced with the γ -IFN retroviral vector. After G418 selection, lethal irradiation, and quality control testing, the lymphokine-expressing autologous tumor cells will be injected back into the patient. The resultant increase in specific immunity against the highly immunogenic gene-modified tumor cells may result in the clearance of the endogenous unmodified metastatic tumors (see Fig 1 for a diagrammatic summary of the approach).

Although the lymphokine gene delivery approach could theoretically be applied to almost any tumor, certain tumor types offer greater chance for success than others. Some of the criteria used to evaluate tumor types include: general availability (tumors that are commonly accessible), tumor cell culturability in vitro, under expression of Class I MHC, known involvement of immune response (especially CTL), and known clinical response to immune therapies. By these criteria, the most logical choice is melanoma. Melanomas are relatively easy to establish as cell lines in

culture (40,41), there is a well established CTL involvement (40,41), many melanomas have low Class I MHC levels (42-44), and they respond to IL-2/LAK/TIL and α -IFN therapies (10). The patient population will be melanoma patients with metastatic disease that has failed other forms of therapy.

3.0 PRE-CLINICAL STUDIES

3.1 SUMMARY

The feasibility of the approach has been tested in several murine tumor model systems using retroviral vectors that express murine γ -IFN. Gene-modified murine tumor cell lines secreted γ -IFN and exhibited substantial increases in the surface expression of Class I MHC proteins. The modified tumor cells were substantially less tumorigenic, apparently due to their ability to invoke increased anti-tumor immunity. Further, injection of γ -IFN-expressing tumor cells resulted in potent CTL activity capable of specifically lysing the unmodified parent tumor cell. Unmodified tumor cells did not engender such a response. Pre-injection with irradiated γ -IFN-expressing tumor cells, but not the unmodified tumor cells, protected animals from subsequent challenge with the unmodified tumor. This effect is presumably due to the observed induction of the tumor specific CTL. These data show that the naturally poor immunogenic properties of tumor cells can be improved by the constitutive expression of γ -IFN delivered by retroviral vectors. The immune system, thus primed, can mount potent responses against the unmodified parent tumor. This conclusion suggests that a similar gene transfer based immunotherapeutic approach to human cancer may be of substantial benefit to cancer patients.

A high titer replication incompetent amphotropic retroviral vector producing cell line which can be used to deliver the human γ -IFN gene to a variety of cell types has been generated. We have used this vector producing cell line to introduce the human γ -IFN gene into a number of human melanoma cell lines. Transduction of human melanoma cells by amphotropic retroviral vectors is efficient and results in the rapid expression of biologically active human γ -IFN. Transduced tumor cell lines show increased expression of human Class I and II MHC (HLA) as determined by protein immunoblotting, immunofluorescence, and fluorescence activated cell sorting (FACS) analysis. γ -IFN-transduced melanoma cells can stimulate CTLs from melanoma patients 5-40X more efficiently than unmodified tumor cells.

3.2 METHODS

3.2.1. Construction of vector producing cell lines

3.2.1.1. CONSTRUCTION OF HUMAN γ -INTERFERON RETROVIRAL PROVECTOR PLASMID, PHU γ -IFN. A cDNA for human γ -IFN was obtained by PCR. RNA was isolated from PHA-stimulated Jurkat T cells by guanidinium thiocyanate extraction followed by ultracentrifugation through a CsCl cushion (45). RNA was reverse-transcribed *in vitro* as described (46) and a gene-specific oligonucleotide pair was used to amplify γ -IFN cDNA by the polymerase chain reaction using Tac polymerase (47). The PCR DNA was repaired with T4 DNA polymerase and Klenow, kinased with T4 Polynucleotide Kinase, and cloned into the *HinC* II site of SK⁺ plasmid (Stratagene) treated with calf intestinal phosphatase. In the sense orientation, the 5' end of the cDNA was adjacent to the Xho I site of the SK⁺ polylinker and the 3' end adjacent to the Cla I site. Sequencing of ph γ -IFN, the retroviral vector, revealed the presence of a one base pair deletion within the ph γ -IFN gene. This deletion was reversed using a multi-step PCR procedure.

The γ -IFN gene in the appropriate orientation was removed from the SK⁺ plasmid by digesting DNA with Xho I and Cla I and cloned into the MoMLV-based retroviral vector, KT-3 (manuscript in preparation). Briefly, KT-3 contains the LTR and packaging sequences from vector N2 (48), except that the ATG translational initiation codon of MLV gag (present in the packaging sequence, Ψ) was mutated to ATT in order to prevent undesired translation of these sequences. The plasmid contains unique Xho I and Cla I cloning sites immediately after the packaging signal and before the Neo^r gene (expressed from the SV40 early promoter). KT 3 plasmid was digested with Xho I and Cla I and ligated with the Xho I-Cla I γ -IFN fragment. Positive clones were identified by restriction mapping. Subsequent sequencing of the γ -IFN cDNA insert in the provector plasmid confirmed the exact identity of the cDNA. The overall structure of Hu γ -IFN provector is described in Fig 2. The sequence and structure of pHu γ -IFN is presented in Appendix H.

3.2.1.2. CONSTRUCTION OF MURINE γ -IFN RETROVIRAL PROVECTOR, PMU γ -IFN. A mouse γ -IFN cDNA cloned into the EcoRI site of pUC18 (49) was obtained. The γ -IFN cDNA was retrieved by EcoRI digestion and the isolated fragment was cloned into the EcoRI site of phosphatase-treated pSP73 vector. The orientation of the cDNA was verified by using appropriate restriction enzyme digestion and DNA sequencing. In the sense orientation, the 5' end of the cDNA was adjacent to the Xho I site of the pSP73 polylinker and the 3' end adjacent to the Cla I site. XhoI-Cla I fragments containing the murine γ -IFN cDNA were retrieved from pSP73 and cloned into the XhoI-Cla I site of KT-3 (see 3.2.1.1 above). See Fig 2 for a diagrammatic representation of the Mu γ -IFN provector.

3.2.1.3 GENERATION OF RETROVIRAL PACKAGING CELL LINES.

MLV structural gene expression vectors. To minimize the possibility of forming RCRV resulting from genetic interactions between the MLV pro-vector DNA and the structural genes of the PCL, separate expression vectors, each lacking the viral LTR, were generated to express the gag/pol (50), amphotropic envelope (51), or xenotropic envelope (52) genes independently. In order to express high levels of the MLV structural proteins in the host cells, the human CMV immediate early promoter was utilized (53). Transcription was terminated by the SV40 late termination signal (residues 2717-2363). A diagrammatic representation of these expression vectors is presented in Fig 3.

Transfection of host cells for the generation of PCLs. A canine cell line, D17 (ATCC #: CCL 183), was co-transfected with a 10X excess of the pSCV10 plasmid (Fig 3) and a methotrexate marker, pFR400 (54). After selection in HAT medium individual clones were isolated and tested for MoMLV gag expression by protein immunoblotting. A gag/pol positive clone was re-transfected with a 10X excess of either amphotropic or xenotropic envelope expression vectors (Fig 2) and a phleomycin resistance marker, pUT507 (55). Co-transfection of a 10X stoichiometric excess of the expression vector over that of a separate selectable marker was done to ensure high copy number of the expression vector in drug resistant cell clones. After selection for transfected drug resistant cells, individual drug resistant cell colonies were expanded and analyzed for MoMLV gag/pol expression by extracellular reverse transcriptase activity (modified from ref 56), intracellular p30^{gag} by Western blot using anti p30 antibodies (goat antiserum #77S000087 from the National Cancer Institute), and either intracellular gp80^{env} (amphotropic envelope) or gp75^{env} (xenotropic envelope) expression by Western blot using anti gp70^{env} (goat antiserum #79S000771 from N.C.I.). Independent clones that expressed high levels of gag/pol and env were transduced with the vector N2, and the clones with best titers that did not generate RCRV were identified. DA and DX cells are the MLV-based amphotropic and xenotropic retroviral PCLs, respectively, identified from these studies.

3.2.1.4. GENERATION OF VECTOR PRODUCING CLONES.

Production of xenotropic pseudotyped vector by transient transfection. Highest titers are obtained when retroviral vectors are introduced into PCLs by transduction instead of transfection (50). Although amphotropic MLV vectors are known to infect the D17 parent cell line, the DA PCL is blocked for infection by amphotropic vector as its receptor is blocked by the expression of amphotropic envelope by "viral interference" (57). To overcome this problem, vectors were used that enter the cell via a different receptor by utilizing another retroviral envelope (xenotropic

envelope). Xenotropic-pseudotyped retroviral vector (58) was generated by calcium phosphate transfection of DX cells with 5 µg of pHuγ-IFN plasmid (described in 3.2.1.1 above). After 2 days, media containing xenotropic vector was collected off the cells and passed through a 0.45 µm filter.

Transduction and selection of retroviral packaging cell line. For transduction, the cells were seeded at 1×10^5 cells/ 6 cm dish with 4 µg/ml polybrene. The following day, 0.2 ml of the supernatant collected off the DX transiently transfected cells (described in 3.2.1.3.above) was used to transduce the Huγ vector into the DA cells. The following day, the media was replaced with fresh media containing 800 µg/ml G418. Cells were grown to confluence and expanded under selection to 10 cm dishes. The cells were then cloned by limiting dilution, clones were screened, and a high titer, retrovirus-free producer clone was identified, VCLHuγ.

3.2.2. Vector Characterization

3.2.2.1. G418 RESISTANCE TITER ASSAY. Titer was determined by addition of serially diluted test samples onto 1×10^5 NIH3T3 or HT1080 cells seeded on a 6 cm diameter tissue culture dish. After 1 day the culture was placed in medium containing G418 and the resistant colonies were enumerated after 10-14 days without correction for cloning efficiency. Titers were routinely determined from at least 2 measurements, and were found to be essentially equivalent for amphotropic vector on the two cell types.

3.2.2.2. CHARACTERIZATION AND QUALITY CONTROL (QC) TESTING. A working cell bank of VCLHuγ will undergo complete characterization and safety evaluation testing, as described in Table I. Vector QC testing is described in Table II. Transduced autologous tumor cell characterization is described in Table III.

3.2.2.3. ASSAY FOR REPLICATION-COMPETENT RETROVIRUS (RCRV) IN VECTOR-CONTAINING SAMPLES. The presence of RCRV was tested in 3 ways: 1) by the standard S⁺L⁻ assay using a mink cell line, M_iCl₁ (ATCC # CCL 64.1), as described (59); or 2) a modification of the S⁺L⁻ test in which test samples were first allowed to replicate (amplified) on the precursor cell line to M_iCl₁, Mv-1-Lu (ATCC # CCL 64), for 14 days before transferring medium to the test cell line, M_iCl₁; or 3) by a marker rescue assay in which test samples were applied to a cell line, MdH, which was generated by delivering a Hyg^r retroviral vector to Mus dunni cells (60). If RCRV is present, then the vector will be rescued from the cell line by trans-complementation, and cell-free supernatants will confer Hyg^r to the target HT1080 cells. In our experience the sensitivities of all of these assays are comparable (1 viral unit per ml) and result in indistinguishable TCID₅₀ titers with positive

murine amphotropic (MA, derived from the cloned hybrid MoMLV-4070A provirus, pAM) (50) retrovirus controls.

3.2.2.4. ASSAY FOR RCRV IN THE VECTOR-PRODUCING CELL LINE, VCLHU γ . Since amphotropic retroviruses would be unable to replicate efficiently on an amphotropic PCL (57), it was possible that very low levels of RCRV existed below the detection limits. To test this we co-cultivated 5×10^5 VCLHU γ vector producing cells with an equal number of Mus dunni cells that are known to efficiently support the replication of all classes of murine RCRVs (60). Every 3-4 days the cell number was split to 5×10^5 . After 10-14 days of this "amplification", the supernatants were removed and tested for RCRV by either the standard S+L- or the MdH assay, described above.

3.2.3. Protein Expression Assays

3.2.3.1. MURINE CLASS I MHC EXPRESSION ASSAYS. RIPA cell extracts were separated on 10% acrylamide gels and blotted onto Immobilon transfer membranes essentially as previously described (61). Detection of Class I MHC proteins in mouse cells was carried out by incubation with a rat monoclonal antibody 7.2.14 (62) and ^{125}I protein A. The epitope detected by the antibody was encoded within a region that is well conserved between murine MHC haplotypes. Alternatively, cells were stained with the 34.4 anti-D^d antibody (63) and binding measured by FACS analysis with fluoresceine isothiocyanate conjugated rabbit anti mouse IgG (Capell).

3.2.3.2. BIOLOGICAL ACTIVITY OF γ -IFN. γ -IFN activity was quantified by measurement of the protective effect against cytotoxic infection with encephalomyocarditis virus (64).

3.3 RESULTS AND DISCUSSION

3.3.1 Retroviral Vector Producing Cell Lines

3.3.1.1 PACKAGING CELL LINES. Due to the safety risks associated with generating amphotropic or xenotropic RCRV by homologous recombination with endogenous sequences, we have developed MLV-based retroviral PCLs from a canine cell line, D17. There are no known canine retroviruses and at least one canine fibroblast cell line Cf2Th, ATCC CRL 1430, has been shown by us to lack genomic sequences homologous with MLV by high stringency genomic hybridization analysis (data not shown). To further decrease the chance of generating RCRV we have generated PCLs which contain genetically unlinked MLV structural genes ("split genome") that have minimal sequence homology with the vector DNA. The resultant requirement for multiple homologous recombinations between the therapeutic pro-vector DNA and the DNA

encoding the MoMLV structural genes ("gag/pol" and "env") present in the PCL, makes the probability of generating RCRV by homologous recombination extremely low.

Multiple clones of cells transfected with gag/pol and env expression vectors were screened by protein immunoblotting (see Methods, 3.2). Individual cell clones were identified that expressed 10-50x higher levels of gag/pol and similar levels of env compared with that of the PCL control PA317 (Fig 4). A number of these potential amphotropic or xenotropic PCLs were tested for their capacity to package retroviral vectors by measuring titer after transduction with the vectors KT-1 and N2. After G418 selection, cell free supernatants were collected from the confluent monolayers and titered by measuring G418^r colonies formed after addition to either NIH3T3 TK- or HT1080 cells. The cell clones with the highest titer were chosen as PCLs and referred to as DA (D17 ampho) and DX (D17 xeno).

In our hands, PA317 generated the highest titer of all the murine PCLs tested (Table IV, experiment 1). By direct comparison, the DA vector titers were as high as or higher than the titer produced from PA317 with both vectors tested (Table IV, experiment 2). In no case was RCRV detected from vector producing cells derived from DA. DA therefore has characteristics (high titer and undetectable RCRV) essential for human gene therapy trials.

3.3.1.2 CHARACTERIZATION OF THE HUMAN γ -IFN VECTOR PRODUCING CELL LINE, VCLHU γ . The γ -IFN proteins are highly species-specific and therefore the human γ -IFN gene must be used for human studies. The clonal vector producing cell line, VCLHu γ , will be carefully characterized with regard to the possible presence of adventitious agents (summarized in Table I). The cell-free supernatant results in an average titer of 3.7×10^6 cfu/ml and several tests have failed to indicate the presence of RCRV. Co-cultivation of the VCLHu γ vector producing cell line with Mus dunni cells (to amplify retrovirus, if present) also fails to detect RCRV. The cell line lacks adventitious agents as determined by broth and agar culture assays (for bacteria, fungi, and mycoplasma) as well as Hoechst staining (for mycoplasma). The cell line appears to have 1 provector with the appropriate genetic structure (Fig 5). Vector from VCLHu γ results in expression of biologically active γ -IFN in transduced cells (Table III) with the appropriate genetic structure (Fig 5). The VCLHu γ master cell bank will be characterized in accordance with the FDA "Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals (1987)". These and other tests are summarized in Table I. The high titer and lack of adventitious agents make VCLHu γ ideal for the production of γ -IFN vector for clinical trials.

3.3.2 Murine Tumor Model Data

3.3.2.1 MURINE γ -IFN VECTOR EXPRESSION AND MHC. The retroviral vector containing the murine γ -IFN gene was generated and introduced into several murine tumor cell lines; L33 (fibrosarcoma), B16F10 (melanoma), CT26 (colo-rectal carcinoma), and Lewis lung carcinoma. All transduced lines produced measurable levels of biologically active γ -IFN detectable in the growth media compared with the non-transduced parent tumor cell lines (Table V). Unmodified CT26, however, displayed a low, but detectable, basal level expression of γ -IFN (Table V). The effect of endogenous γ -IFN expression on MHC Class I protein in L33, B16F10 and CT26 was determined by protein immunoblotting. With the exception of CT26, a large increase (>20-fold) in the level of MHC was observed in all γ -IFN-vector-transduced tumor cell lines (Fig 6). CT26 displayed relatively high basal levels of MHC, possibly due to the basal level of γ -IFN produced by this cell line (Table V). Consequently, CT26 displayed only a modest (2-3-fold) increase in MHC expression (Fig 6A). FACS analysis indicates that the Class I MHC was cell surface associated (Fig 7).

Transduction by murine γ -IFN vector thus substantially increases the level of Class I MHC. Similar reports have been reported by other investigators (30). Since these proteins present antigen to the immune system, the effect of vector transduction should be to improve immune responses, especially those that are Class I restricted such as CTL.

3.3.2.2. TUMORIGENICITY OF MURINE γ -IFN-EXPRESSING CELLS. Improved immune responses against γ -IFN-transduced tumor cells might be reflected in a decrease in the tumorigenic properties of the tumor. The effect of endogenous γ -IFN expression on the tumorigenic properties of the vector modified tumor cells was therefore assessed in syngeneic animals. Vector-modified L33 (Fig 8A), B16F10 (Fig 8B), CT26 (Fig 8C), and Lewis lung (Fig 8D) were all substantially less tumorigenic than the corresponding unmodified parent tumor cell lines (Fig 8).

In additional experiments, recombinant γ -IFN protein added to the L33 or B16F10 cells did not affect tumorigenicity (Fig 8), even though control experiments indicate that treatment resulted in high level MHC expression for as long as 3 days after treatment (data not shown). This indicates that the effect of constitutive expression of γ -IFN obtained by gene transfer can be superior to the effect obtained by treatment with recombinant γ -IFN protein. Control experiments with vectors which express only Neo^r did not affect tumorigenicity (data not shown), indicating that the effect is due to γ -IFN expression per se and not due to the vector transduction process in general.

The tumorigenic properties of L33 and B16F10 γ -IFN-expressing tumor cell lines were also tested in immunocompromised athymic nude mice. The L33 tumors grew almost as well as the unmodified tumor (Fig 9), indicating that the decreased tumorigenicity observed in normal animals was at least partly due to an immune response (most likely a T cell). B16F10, however, also failed to grow as a tumor even in nude mice (Fig 9), indicating that γ -IFN has additional effects on the tumor cells besides those that are T cell mediated.

These data suggest that there are at least two mechanisms for the decreased tumorigenicity associated with γ -IFN-transduced tumor cells: 1) T cell mediated immunity and 2) a non-T cell effect, due either to other immune cells (e.g., macrophages or NK cells) or to a direct inhibition of tumor growth.

3.3.2.3. GENERATION OF TUMOR-SPECIFIC CTL. The immunological mechanisms for the decreased tumorigenicity of tumor cells expressing γ -IFN were of interest. There were at least three possible explanations for immune protection observed for γ -IFN-expressing, but not unmodified, tumor cells. Either 1) the unmodified cells were not capable of eliciting cytotoxic lymphocyte responses, or 2) tumor-specific cytotoxic lymphocytes exist but the cells were resistant to lysis, or 3) both. The exceptionally low levels of Class I MHC on most tumor cells (see Figs 6,7) and the need for Class I MHC for antigen presentation (3, 4) suggested that immune responses against the γ -IFN-expressing tumor cells might not have been effective against the unmodified tumor.

To test these possibilities, the CTL induction characteristics of unmodified and γ -IFN-expressing tumor cells were compared, using the unmodified tumor cell as a target. The data in Fig 10A indicate that injection of B16F10 irradiated tumor cells resulted in no substantial CTL response. However, when mice were injected only once with γ -IFN-expressing B16F10, substantial CTL responses were observed. Additionally, the CTL response was greater when the mice were immunized twice (Fig 10B). Furthermore, CTL induction was not substantially different whether the γ -IFN-expressing B16F10 cells were injected intraperitoneally or intramuscularly (Fig 10C). The level of class I MHC observed for B16F10 therefore appears to present antigen sufficiently well to act as a target for, but not as an inducer of, CTL.

Similarly, the data in Fig 11 indicate that injection of unmodified CT26 irradiated tumor cells resulted in no substantial CTL response. Injection with γ -IFN-expressing CT26, however, resulted in measurable CTL responses (Fig 11A). CTL induction was also tested in animals receiving unirradiated tumor cells. The data in Fig 11B indicate that γ -IFN-transduced CT26 elicit more potent anti-tumor cytolytic activity than unmodified CT26. Control experiments with the

unrelated cell lines, BC10ME and B16F10, indicated that the anti-CT26 immune response was specific (Fig 11C).

A few animals injected with unirradiated γ -IFN-expressing CT26 cells generated small tumors while most animals did not generate tumors (Fig 8C). The splenocytes collected from tumor-free vs. tumor-bearing animals were tested *in vitro* for anti-CT26 CTL activity. The data (Fig 11D) indicate that the level of anti-tumor CTL activity is substantially higher in tumor-free animals than that observed for animals with tumors. These data suggest that the tumor cells failed to form tumors as a result of more potent CTL activity. This confirms the importance of CTL for inhibiting tumor growth.

Substantial CTL induction was also observed after injection of Lewis lung carcinoma or the corresponding γ -IFN-transduced tumor cells (Fig 12A). The anti-tumor response was specific, as determined by the inability of anti-LLT cytolytic lymphocytes to lyse the unrelated tumors, B16F10 and CT26 (Fig 12B).

These data show that in all three models the lack of measurable response against the unmodified tumor is due to insufficient CTL induction, not due to the inability of the cell to be lysed by CTL. These results have been confirmed by other investigators with another tumor model (30).

3.3.2.4. MURINE TUMOR VACCINATION STUDIES. To determine whether the observed immune responses could protect mice from challenge with unmodified tumor cells, vaccination studies were performed by priming mice with either unmodified, or γ -IFN-transduced, irradiated B16F10 tumor cells. The animals were then challenged with the unmodified parental tumor cell. The data in Fig 13A indicate that a single pre-injection with as little as 4×10^5 γ -IFN-expressing tumor cells, but not unmodified tumor cells, resulted in a measurable (~ 2.5 -fold) protection against tumor challenge. A single injection with larger numbers of cells resulted in even greater (~ 5 -fold) protective immunity (Fig 13B). Once immunity had been induced by vector-modified cells, the resultant immune effectors react not only against the γ -IFN-expressing cell (resulting in decreased tumorigenicity, Fig 8) but also against the unmodified tumor cell (resulting in the immune protection against unmodified tumor, Fig 13). γ -IFN-transduced tumor cells are thus capable of generating systemic, protective, anti-tumor immunity.

3.3.3. Human Melanoma Data

3.3.3.1. TRANSDUCIBILITY OF HUMAN MELANOMA CELLS *IN VITRO*. To determine how effectively human melanoma cell lines could be transduced with retroviral vectors, we utilized a vector identical with Huy vector, except that the E. coli β -galactosidase gene is expressed instead of

γ -IFN (CB β -gal). This allowed the transduction efficiency to be determined by enumeration of blue cells, after staining with X-gal (65). The data in Fig 14 indicate that three different human melanoma cells are all readily transduced with amphotropic retroviral vector.

3.3.3.2. γ -IFN AND HLA EXPRESSION IN HU γ VECTOR-TRANSDUCED HUMAN MELANOMA CELLS. Hu γ retroviral vector was used to introduce the human γ -IFN gene into a number of short term melanoma tumor cell lines. Cell-free supernatants from G418 selected transduced human melanoma cell lines were tested for γ -IFN activity. The data in Table VI indicate that biologically active γ -IFN is expressed in transduced cells.

To determine the effect of gene transfer of γ -IFN on human Class I MHC (HLA) expression, cell extracts were prepared and analyzed for HLA expression by protein immunoblotting. The data in Fig 15 indicate that transduction of human γ -IFN vector into three different human melanoma cell lines resulted in a 2-7-fold increase in the level of MHC (HLA I), as determined by protein immunoblotting. Immunofluorescence indicates that this is surface associated (data not shown). These stably transduced G418 resistant tumor cells were then examined by flow cytometry for changes in the expression of HLA Class I and II encoded gene products as indicated by W6/32 and L243 staining, respectively. Representative data for two such lines (Table VII) demonstrate the substantial increases in the level of cell surface expression of Class I HLA proteins following transduction. Although the percent positivity of the two representative cell lines for Class I HLA is not measurably changed relative to the parental cells, the level of expression in gene modified DM6 of these important proteins is increased 1.6-fold and by gene modified DM265 by more than 2.6-fold. With respect to Class II HLA proteins, gene modified DM6 tumor cells have a 5-fold increase in percent positivity. Gene transduced DM265 tumor cells demonstrate a small increase in frequency of positives but a measurable 1.4-fold increase in the level of expression of Class II proteins. The data indicate that transduction of these tumor cell lines (and other such tumor cells not shown) results in substantial and long lasting upregulation of both Class I and Class II HLA proteins.

3.3.3.3. RECOGNITION OF GENE MODIFIED MELANOMA BY HLA RESTRICTED, MELANOMA SPECIFIC, CYTOTOXIC T-CELLS. We have examined the functional nature of the increased HLA proteins on the gene transduced tumor cell lines. DM6 and DM6/ γ -IFN tumor cells were tested *in vitro* as stimulator cells in a ^3H -thymidine uptake assay using an HLA-A2 restricted, melanoma specific cytotoxic T-cell line as a responding population. While native DM6 tumor cells elicit a measurable response, the gene modified DM6/ γ -IFN are dramatically more effective (6- to 40-fold) in triggering the T-cells (Table VIII). These data (Table VIII) clearly demonstrate the superiority of γ -IFN enhanced DM6/ γ -IFN to elicit a T-cell proliferative response.

DM6 parental or γ -IFN transduced tumor cells were also tested as target cells using an HLA-A2 restricted, melanoma specific cytotoxic T-cell line. These HLA Class I restricted effector cells showed 161 lytic units (35%) per 10^6 cells of activity against the parental DM6 line and 270 lytic units of activity against the gene transduced tumor line, an increase of 67% (Table IX). The marginally increased T-cell recognition of DM6/ γ -IFN may be related to the enhanced expression of T-cell defined tumor associated antigens presented in the context of the increased Class I HLA proteins. Consistent with this is the dramatic upward shift in the inhibition curve of antibody W6/32 in blocking studies using parental or IFN gene transduced DM6 as target cells (Figure 16). In this study, the same quantity of W6/32 reduced the lysis of DM6 by 61% but reduced lysis of DM6/ γ -IFN by only 16%. The data indicate that the increased HLA Class I antigens on the gene transduced tumor cells are fully functional to present melanoma associated, T-cell defined tumor antigens required for T-cell recognition.

3.4 CONCLUSIONS

Data from animal tumor models showed that the tumorigenic phenotype was, at least in part, due to poor anti-tumor immunity. The available evidence strongly suggests that the lack of effective immunity is due to poor CTL induction and not the inability of a tumor cell to act as a target for CTL lysis. Our *in vitro* studies with human melanoma immune responses showed a similar effect, since γ -IFN transduction substantially increased CTL proliferative responses but did not dramatically improve the ability of the tumor cell to act as a target (Table VIII). The parallel between *in vivo* murine studies and *in vitro* human studies suggests that CTL responses in melanoma patients may be dramatically stimulated clinically by the administration of γ -IFN transduced autologous tumor cells and that such responses could effectively recognize and destroy melanoma cells.

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**A PHASE I TRIAL OF HUMAN GAMMA INTERFERON TRANSDUCED
AUTOLOGOUS TUMOR CELLS IN PATIENTS WITH DISSEMINATED MALIGNANT
MELANOMA**

4.1 OBJECTIVES:

The primary objective of this protocol will be to evaluate safety, biological response, and survival in patients serially treated with autologous melanoma cells transduced with the gene for human gamma interferon (γ -IFN). We will monitor the safety and toxicity of the treatment and will attempt to determine its immunological effects on the patient. We will also evaluate the clinical responses and duration of any such response to this treatment.

4.2 INTRODUCTION AND BACKGROUND:

The incidence of melanoma is steadily increasing. Cutaneous melanoma is second only to carcinoma of the lung in females in terms of increasing incidence. During this decade, approximately 1 in 90 Caucasian Americans will develop malignant melanoma.

At the present time, the disseminated form of malignant melanoma is resistant to all conventional types of therapy. The ineffectiveness of hormonal therapy and chemotherapy has led investigators to evaluate a variety of immunotherapy regimens. The ultimate goal has been to consistently augment the immunological reactivity of melanoma patients to their own tumor. Active specific immunotherapy (treatment with autologous tumor cells) has been shown to be effective in some patients with metastatic melanoma. Berd et. al (1) immunized patients with irradiated autologous tumor cells and noted clinical responses in 25% of the patients treated. Other investigators have reported similar response rates in patients with renal cell carcinoma and colon carcinoma (2-4). Mitchell et. al. (5) has reported clinical responses and stimulation of specific cytotoxic T-lymphocytes (CTL) in patients immunized with autologous melanoma cells. Seigler et. al. (6-8) have serially injected high risk Stage I and Stage II melanoma patients using either x-irradiated melanoma cells or a vaccinia viral oncolysate of the tumor cells and have demonstrated improved survival

in patients receiving adjuvant active treatment.

During the past 3 years, we have demonstrated that specific CTL can be generated *in vitro* by exposing patient lymphocytes to autologous tumor cells in the presence of a low dose interleukin-2 (9). The specific CTL have effected cell mediated killing both *in vitro* and *in vivo* (10,11). The killing is major histocompatibility complex (MHC) restricted and can be generated from either peripheral blood lymphocytes or lymph node cells. Class I MHC proteins are involved in the presentation of intracellular antigens to the immune system. Thus, suboptimal levels of MHC membrane proteins may prevent effective presentation of tumor associated antigens.

Recently, methods for improving anti-tumor immune responses in cancer patients by gene transduction of tumor cells have been proposed. A variety of lymphokine genes have been introduced into tumor cells using retroviral vectors (12-18). The transduced cells express the inserted gene and secrete the specific lymphokine. We have recently shown that human melanoma cells transduced with the gene for human γ -IFN serve as potent stimulators of specific CTL (unpublished data). The γ -IFN gene is expressed and results in a substantial increase in the levels of Class I MHC protein. The MHC restricted stimulation of tumor-specific CTL is markedly enhanced. Gene-altered autologous melanoma cells might well provoke substantial post-immunological responses against host tumor burden.

4.3 PATIENT SELECTION:

Eligibility Criteria:

- A. Histologically confirmed diagnosis of metastatic melanoma.
- B. The patient must have one or more measurable lesions remaining after removal of the lesion used to prepare the cell suspension.
- C. Estimated life expectancy of at least 6 months.
- D. Age of at least 18 years.
- E. Signed informed consent.
- F. Complete recovery from effects of prior major surgery before initiating study participation.
- G. Adequate hematologic, renal, and hepatic function.

Exclusion Criteria:

- A. Medical or psychological impediment to probable compliance with the protocol.
- B. Pregnant or lactating women. Women of child bearing potential must have a negative pregnancy test prior to entrance into the study.
- C. Male and female participants must commit to use effective contraception for the duration of the study.
- D. Concurrent chemotherapy, radiation therapy or immunotherapy.
- E. Concurrent treatment with corticosteroids or treatment with corticosteroids within 1 week of first treatment.
- F. Concurrent second malignant tumor other than in situ carcinoma of the cervix.
- G. Autoimmune diseases such as, but not restricted to, systemic lupus erythematosus, multiple sclerosis or anklosing spondylitis.
- H. HIV positive.

4.4 PRE-STUDY EVALUATIONS:

- A. History and physical exam within 2 weeks prior to first treatment.
- B. Diagnostic procedures to be done within 2 weeks prior to first treatment include:
 - (1) Complete blood counts and liver function studies;
 - (2) Electrocardiogram;
 - (3) Baseline blood draws for immunological testing;
 - (4) Scans and x-rays include:
 - a. Staging computerized tomography of the head, chest and abdomen within 4 weeks of first treatment;
 - b. Chest x-ray within 4 weeks prior to the first treatment. All scans and x-rays will be used to document measurable or evaluable lesions.

4.5 REGISTRATION:

Registration is defined as the date of entry onto the study and requires that the patient has met all eligibility criteria, has no exclusion criteria, and has signed a consent form.

4.6 STUDY DESIGN:

This Phase I study will be conducted exclusively at Duke University Medical Center. The study will enroll up to 20 patients. All patients will be evaluated for immune responses and survival, although not all these evaluations will be measurable end points. The primary end point will be evaluation of the toxicity of the treatment. A grade 3 or 4 will eliminate the patient from continuing on study as noted in appendix G.

An additional endpoint will be the assessment of CTL responses. Peripheral blood mononuclear cells (PBMC) sampled both before and after product administration will be assayed for cytolytic activity on unmodified autologous melanoma target cells. CTL activities will be determined and converted to lytic units. Doubling of CTL lytic units above control pre-treatment levels will be considered an endpoint. Following enrollment of an initial 10 patients, if none attain this endpoint, there is a 95% chance that less than 30% of patients in this population subset will engender such a response, and, barring clear objective evidence of tumor regression, the protocol will be discontinued. If increases in CTL lytic units are observed in at least one of the initial 10 patients, an additional 10 patients will be enrolled. This will allow for estimation of toxicity with a standard error of 7%.

Survival time from the date of diagnosis of metastatic melanoma to death will be evaluated. Also, the time from the date of initiation of therapy on this study to death or 36 months, will be measured. Therapy will continue until the end of the 21 months for vaccinations; however, a patients will be taken off study at the time of progression of disease, death of the patient, or discretion of the investigator.

4.7 TREATMENT SCHEDULE AND ON-STUDY EVALUATION:

A. γ -IFN Transduced autologous tumor (ITAT) dosage and schedule:

The patients will be administered approximately 2×10^6 tumor cells that have been transduced with the γ -IFN gene and irradiated with 10,000 RADS. ITAT will be administered every 2 weeks for the first 3 months. Subsequent boosting may be administered monthly thereafter until 24 injections have been administered.

B. Immunological tests:

- a. On at least 2 occasions before treatment, 20 ml. of heparinized blood

and 20 ml. of unheparinized blood for baseline immunological studies will be obtained. Antibody mediated immunity to melanoma will be measured by enzyme immunoassay and complement-dependent cytotoxicity.

- b. Cell mediated immunity to unmodified autologous melanoma target cells will be measured using a chromium release assay.

4.8 SCHEDULE FOR TUMOR MEASUREMENTS:

- A. Measurements of evaluable lesions will be made prior to therapy and every other week for 3 months and monthly thereafter.
- B. X-rays of evaluable lesions will be taken at weeks 1, 8 and 16; CT scans of evaluable lesions will be completed at weeks 8 and 16 and additional scans will be obtained as clinically indicated.
- C. Additional tumor measurements will be made of all objective partial or complete responses.

Definitions for patient responses:

CR — COMPLETE RESPONSE

During an observation, no disease is apparent and no evidence of disease is observed for at least 28 days following the preceding evaluation.

PR — PARTIAL REPNSE

A 50% or greater decrease from baseline in overall tumor size is noted, and no progressive disease is observed for at least 28 days following the observation of the 50% decrease.

SD — STABLE DISEASE

The patient has stable disease if he/she does not exhibit at least a 50% decrease in the overall size of all lesions. In addition, no new lesions may have appeared.

PD — PROGRESSIVE DISEASE

Progressive disease will be measured with reference to baseline values unless a decrease in size has been observed. A 50% or greater increase in the size of any single lesion or the appearance of a new lesion constitutes progressive disease.

4.9 DISCONTINUATION OF THE ACTIVE IMMUNIZATION SCHEDULE:

- A. Therapy will be discontinued if grade 3 or 4 toxicity occurs.
- B. Therapy will be discontinued if the patient refuses further treatment for any reason, including intolerable subjective toxicity.
- C. Therapy will be discontinued if the disease progresses sufficiently to cause symptoms that require other treatments.
- D. Therapy will be discontinued for patients who have continued rapidly progressive disease.
- E. Following the enrollment of an initial 10 patients, if 0/10 demonstrate an increase in CTL above pretreatment levels, and barring objective evidence of tumor regression, the protocol will be discontinued.

4.10 TOXICITY:

- A. All toxicities and adverse reactions will be recorded in the case report form. All adverse reactions will be reported to the Institutional Review Board.
- B. Possible immediate side effects from the injections may include allergic reactions such as rash, skin tenderness, swelling, fever, or hives. Also possible at the site of injection is granuloma (lump) formation. Rarely, more severe allergic reactions could occur, such as severe shortness of breath, low blood pressure or fainting. The transduced autologous tumor cells could conceivably contain viruses undetectable with current testing procedures which could be infectious.
- C. The Investigator will observe good clinical practice in the assessment and management of toxicity to treatment.

4.11 PATIENT FOLLOW UP:

- A. All patients will be followed for 36 months from the time of first treatment or until death.
- B. If deemed necessary to monitor the duration of an objective clinical response,

a CT scan will be performed every 3 months during the first year and every 4 months during the second year and every 6 months during the third year.

4.12 PROCEDURE FOR REPORTING ADVERSE REACTIONS:

- A. Death of any patient receiving treatment, even if the death does not appear to be related to the treatment.
- B. Any serious (life threatening) and unexpected adverse reaction in a patient receiving treatment, even if the reaction is not clearly related to the treatment.
- C. The Principal Investigator will notify the Institutional Review Board, the Recombinant DNA Advisory Committee (RAC), and the FDA of any serious adverse effects within the following time frames:
 - 1. Written report within 10 working days of all adverse reactions that are serious and unexpected.
 - 2. Within 3 days the FDA will be notified by telephone of any unexpected death or life threatening experience.All adverse reactions will be entered on the case report form.

4.13 STUDY MONITORING:

All records pertaining to the study will be kept on file following conclusion of the study. All case report forms, including pre-study, on-study and off-study forms will be available for review by the FDA. Survival status, time and location of disease progression, and additional treatment will be reported quarterly on post-study follow up forms.

4.14 INFORMED CONSENT:

Approved informed consent forms will be signed and witnessed for each patient entering the study prior to initiation of treatment. The forms will be kept with the case report forms and copies submitted to the clinical monitor.

4.15 INSTITUTIONAL REVIEW BOARD:

Prior to initiating the study, the Principal Investigator will obtain and comply with all requirements of his respective IRB committee. The IRB will be informed of all

protocol changes and will receive safety reports. Prior to initiating the study, the Principal Investigator will obtain approval from both the FDA and the NIH Recombinant DNA Advisory Committee (RAC).

4.16 "ITAT" PREPARATION AND STORAGE:

Autologous tumor surgically obtained from patients will be utilized to establish short term tumor cell cultures. The resultant cell culture will be transduced with the γ -IFN retroviral vector construct. The transduced tumor cells will be subjected to standard microbiological biological testing as well as evaluated for mycoplasma. After lethal irradiation, the γ -IFN-expressing tumor cell will be appropriately aliquoted and maintained at -135°C in sealed vials. They will then be thawed for re-injection into the same patient. The retroviral vector used in these studies is based upon Moloney murine leukemia virus, which has been engineered to replace its extraneous viral coding sequences with the human γ -IFN cDNA. Unlike true viruses, the recombinant vector is incapable of replication since it lacks all of the viral structural genes. A detailed characterization of the vector gene construct will be presented to the RAC and the FDA for approval prior to initiation of the study.

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5.0 INFORMED CONSENT FORM

A PHASE I TRIAL OF HUMAN GAMMA
INTERFERON TRANSDUCED AUTOLOGOUS
TUMOR CELLS in PATIENTS with
DISSEMINATED MALIGNANT MELANOMA

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IRB# 1456 92-12

Your doctor has explained to you that you have advanced malignant melanoma which cannot be cured by currently available standard treatments (surgery, chemotherapy, hormonal therapy or radiation therapy). You are being asked to participate in a research study which was designed to test the safety and effectiveness of an investigational treatment for melanoma.

This treatment utilizes an experimental substance which is produced at Duke University Medical Center and at Viagene Corporation. The treatment is prepared by surgically removing one of the metastatic melanoma's from your body and processing this tumor in the lab in a test tube. The tumor cells in the test tube will be altered or changed by inserting a gene (genetic material) which causes the tumor cells to make a protein called gamma Interferon (γ -IFN). These cells are called gamma interferon transduced autologous tumor cells.

This gene is inserted into your tumor cells by an altered virus (vector) which does not grow but is intended to act as a vehicle to transfer the gene into the tumor cells. Once the virus gets the gene into the tumor cells, these cells will hopefully produce gamma Interferon and will be made into a treatment solution to be given to you by injection.

This research study is designed to determine whether this solution is safe and can perhaps stimulate your body to reject your melanoma.

If you chose to enter this program, you will be given a vaccination underneath the skin (subcutaneously) of your body every two weeks for three months and monthly thereafter until you receive twenty-four (24) total treatments. This will require twenty-one (21) months.

Patients Initials

A PHASE I TRIAL of HUMAN GAMMA
INTERFERON TRANSDUCED AUTOLOGOUS
TUMOR CELLS in PATIENTS with
DISSEMINATED MALIGNANT MELANOMA

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Extra blood tests will be done because of this research. The blood will be obtained by venipuncture (needle stick in a blood vessel in the arm). This is the standard method for obtaining blood and is momentarily painful. There is a small risk of fainting, of some bruising (bleeding under the skin), and a rare (1/1000) risk of infection. The blood will be drawn at each treatment with a total of 300 cc. over the twenty-one (21) months of this study. You will be followed annually after the completion of the study for progress. This follow up will continue for the rest of your life.

SIDE EFFECTS: It is possible that this vaccination may cause allergic reactions, such as rash, skin tenderness, swelling, fever or hives. In addition, you have approximately a 1/5 chance of developing a lump at the injection site(s). These lumps may become hard and inflamed or may become ulcerated or require draining by your physician. The lumps may persist as long as you are receiving the treatment; however, they should resolve within a few weeks to months after discontinuing therapy. Rarely, a more severe allergic reaction could occur, such as severe shortness of breath, low blood pressure or fainting. It is also possible that you could get a viral infection which may cause serious illness. In addition, the treatment solution could contain viruses which may have not been identified in our tests. Receiving injections of these solutions could also result in other serious side effects of which we are currently unaware. Indeed, unknown side effects are possible as this treatment preparation has not yet been tested in humans. The side effects may be experienced in varying degrees. Any of the above side effects requiring hospitalization for management intervention will necessitate removal from the treatment program. You will be asked to contact Dr. Seigler at (919) 684-3942, should any side effects occur or should you have any other questions/problems at any time during this treatment.

Patients Initials

A PHASE I TRIAL OF HUMAN GAMMA
INTERFERON TRANSDUCED AUTOLOGOUS
TUMOR CELLS in PATIENTS with
DISSEMINATED MALIGNANT MELANOMA

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There is no guarantee that this treatment will have any beneficial effects against your tumor.

There is a 50/50 chance that your surgically removed tumor cells will grow in culture in the test tube and incorporate the gene from the virus. Only if such incorporation into cells and production of the Interferon substance occurs can a suitable injection liquid be prepared and the treatment program take place. If incorporation of the virus and gene into your tumor does not occur, you will not be able to continue with the study.

POTENTIAL BENEFITS: There may be no benefit to you, but it is hoped that the knowledge gained from the experiment may help other patients in the future.

FOR WOMEN OF CHILDBEARING POTENTIAL: Since this research may have an adverse reaction on an unborn child and should therefore not be done during pregnancy, it is necessary that a pregnancy test (using two teaspoonfuls of blood drawn from a vein by needle stick) be done first. You will be asked to take contraceptive measures while you are participating in this study.

FOR MEN WITH POTENTIAL TO FATHER CHILDREN: It is not yet determined whether this treatment can adversely affect the sperm development in men and thus have an adverse effect on an unborn child. Therefore, men who are sexually active should take appropriate contraceptive measures for the duration of this research.

ALTERNATIVE TREATMENTS: Alternative treatments for your disease include chemotherapy drugs, standard or investigational or other more investigational therapies. At any time you may choose to receive only supportive care with symptomatic treatment for relief of your discomforts.

Patients initials

A PHASE 1 TRIAL OF HUMAN GAMMA
INTERFERON TRANSDUCED AUTOLOGOUS
TUMOR CELLS in PATIENTS with
DISSEMINATED MALIGNANT MELANOMA

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COSTS: The treatment and all experimental costs will be supplied free of charge by the study sponsor. Any other cost including costs of needed medical care will be the responsibility of you and/or your insurance carrier. You may contact our Business Office for further information at (919) 684-2395.

Taking part in this study is entirely voluntary. You may decline to participate or you may withdraw your consent and discontinue participation in the project at any time without affecting your needed medical care or your relationship with your physician. If your physician determines it is not in your best interests to continue participation in this study, your physician may discontinue this treatment. You will be given a copy of this informed consent. Any questions or concerns you may have about this study will be answered by the Principal Investigator, Dr. Hilliard Seigler, (919)684-3942.

During your treatments, you will also be notified of any additional information that becomes available and which might influence your decision to continue in the study.

Immediate necessary care is available if an individual is injured because of participation in a research project. However, there is no provision for free medical care or for monetary compensation for such injury. Further information concerning this and your rights as a research subject can be obtained from the Hospital Risk Management Office (919)684-3277.

The confidentiality of this study's records identifying you will be maintained within Duke University Medical Center. Your identity will remain confidential if material from the record is used for publication or for educational purposes. However, you should be aware that the sponsor, Viagene, the Food and Drug Administration, and/or the National Cancer Institute may inspect the records.

Patient's Initials

A PHASE I TRIAL of HUMAN GAMMA
INTERFERON TRANSDUCED AUTOLOGOUS
TUMOR CELLS in PATIENTS with
DISSEMINATED MALIGNANT MELANOMA

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In the event of your death, even if it is unrelated to your cancer (e.g. accident or other illness), an autopsy will be requested. You can advise your next of kin of your wishes and you or your family have the right to refuse without interfering with your participation in this research project or needed medical care.

"I have read the above and have been given the opportunity to discuss it and to ask questions. I have been informed that I may contact Dr. Seigler at (919)684-3942 to answer any questions that I may have during the investigation. I agree to participate as a research subject and certify that I have been informed of the purposes, procedures, risks and possible benefits of this study. I agree to participate with the understanding that I may refuse to participate and may withdraw at any time without interfering with my regular medical care."

Signature of Patient

Date

Signature of person obtaining consent

Date

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME	POSITION TITLE		
Hilliard F. Seigler, M.D.	Professor of Surgery, Professor of Immunology		
EDUCATION (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)			
INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
University of North Carolina-Chapel Hill, NC	B.A.	1956	Chemistry
University of North Carolina-Chapel Hill, NC	M.D.	1960	Medicine
Duke University-Durham, NC (Post-Doctoral fellow)			Immunogenetics

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

PROFESSIONAL EXPERIENCE:

1983-present Present Editorial Board, Annals of Surgery
 1981-present Professor of Immunology, Duke University Medical Center, Durham, N.C.
 1981-present Research Professor, Yerkes Regional Primate Center, Emory University, Atlanta, GA
 1977-present Professor of Surgery, Duke University Medical Center, Durham, N.C.
 1971-1977 Associate Professor of Immunology, Duke University Medical Center, Durham, N.C.
 1971-1972 Chief of Surgery, Veterans Administration Hospital, Durham, N.C.
 1967-1970 Assistant Professor of Surgery, Duke University Medical Center, Durham, N.C., Clinical Investigator, Veterans Administration Hospital, Durham, N.C.
 1965-1967 Research Fellow of Immunogenetics, NIH Special Fellowship, Duke University medical Center, Durham, N.C.
 1964-1965 Chief Resident in Surgery, University of North Carolina, Chapel Hill, N.C.

PUBLICATIONS:

From 240 publications

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HUMAN GENE TRANSFER PROTOCOL

9306-044 Deisseroth, Albert B., Kavanagh, John, and Champlin, Richard, University of Texas MD Anderson Cancer Center, Houston, Texas; *Use of Safety-Modified Retroviruses to Introduce Chemotherapy Resistance Sequences into Normal Hematopoietic Cells for Chemoprotection During the Therapy of Ovarian Cancer: A Pilot Trial.*

Date of RAC Approval: 6/7/1993

Date of NIH Approval: 12/2/1993

SCIENTIFIC ABSTRACT

Replication incompetent, recombination incompetent retroviral vectors will be used to introduce chemotherapy resistance cDNAs into the normal stem cells of autologous peripheral blood and marrow cells removed and stored following chemotherapy delivered to patients with ovarian cancer who are poor risk (second look positive), and therefore at very high risk of relapse (80%). We estimate that between 0.6 and 2×10^6 CD34 positive cells/kg will be infused and that among these, 10% of the 30% of the total cells infused will be exposed to a MDR-1 containing vector and therefore will be modified with the MDR-1 cDNA. Therefore, 2,000 and 200,000 cells will be marked with MDR-1 cDNA in the autologous cells used for transplant. We will look for the number of MDR-1 marked cells using a methylcellulose late progenitor colony culture system and a PCR assay for the MDR-1 gene used previously. In addition, we will monitor the acquisition of chemotherapy resistance by stem cells of varying degrees of immaturity by using culture assays for these cells under chemotherapy selection (methylcellulose assay and colonies grown from Dexter cultures for more than 35 days, using PCR, antibodies for gp170, and functional assays for the efflux pump coded for by MDR-1). These studies will help us evaluate if introduction of MDR-1 cDNA into peripheral blood or marrow cells will confer chemotherapy resistance on these cells, thus allowing therapy of a greater level of intensity to be delivered and therefore change the course of poor prognosis ovarian cancer.

NON-TECHNICAL ABSTRACT:

Autologous bone marrow transplantation is a technique which makes safe the very high doses of chemotherapy and radiation which are required to eradicate some populations of leukemia cells. The marrow is removed from the hip bones of the patient at the time of remission induced by conventional dose chemotherapy, stored and re-infused into the patient after intensive therapy in order to restore marrow function. Peripheral blood will also be used for the restoration of hematopoietic function following transplantation. Although the responses seen of metablatic and advanced ovarian cancer in poor prognosis patients is often dramatic following the intensive therapy used in the bone marrow transplantation, patients who respond do not exhibit increased survival. This indicates that additional therapy following recovery from the transplant might be beneficial. In order to make possible the delivery of relatively intensive doses of Taxol following autologous transplant, we will introduce the MDR-1 cDNA into the stem cells of patients with ovarian cancer before storage for transplant. To accomplish this, a portion of the bone marrow and peripheral blood cells stored from patients will be incubated with the marking vector. This vector will introduce a MDR-1 cDNA into these neoplastic cells. If the neoplastic cells contain the marker, then they will exhibit increased levels of resistance to the unmodified cells. The establishment of this marker in the cells will promote their growth over unmodified cells of conventional dose chemotherapy if given following the autograft. If this is the case, we will be in a position to test high dose Taxol delivered after autologous transplant in ovarian cancer and alter the unfavorable natural history of this disease. The results of this study will be used to improve the therapy given to patients with ovarian cancer. It is not designed to benefit the patients themselves.

APPENDIX A

THE UNIVERSITY OF TEXAS
M.D. ANDERSON CANCER CENTER
Division of Medicine

Protocol Title: Use of safety-modified retroviruses to introduce chemotherapy resistance sequences into normal hematopoietic stem cells for chemoprotection during the therapy of ovarian cancer: A Pilot Trial

- 1.0 OBJECTIVES
- 2.0 BACKGROUND
- 3.0 BACKGROUND DRUG INFORMATION
- 4.0 PATIENT ELIGIBILITY
- 5.0 TREATMENT PLAN
- 6.0 PRE-TREATMENT EVALUATION
- 7.0 EVALUATION DURING STUDY
- 8.0 CRITERIA FOR RESPONSE AND TOXICITY
- 9.0 CRITERIA FOR REMOVAL FROM STUDY
- 10.0 STATISTICAL CONSIDERATIONS

Protocol

REFERENCES

- APPENDIX A1: Zubrod Performance Scale
- APPENDIX A2: Cardiac Status Scale
- APPENDIX B: Ceprate Stem Cell Collector
- APPENDIX C: Background Drug Information
- APPENDIX D: Preparation and Conditions of Exposure of Retrovirus
- APPENDIX E: Toxicity Criteria
- APPENDIX F: Evaluation Before and During Therapy

CONSENT FORM

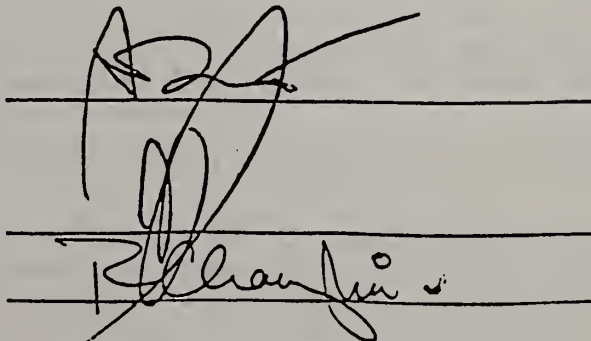
Study Chairman:

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Study Co-Chairman:

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Study Co-Investigators:

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PROTOCOL ABSTRACT

Protocol: (Give number and abbreviated title) (Two lines not to exceed 75 characters per line)

Introduction of Chemotherapy Resistance into Normal Marrow Cells

STUDY CHAIRMAN:

Albert Deisseroth, M.D., Ph.D., John Kavanagh, M.D.
and Richard Champlin, M.D.

Patient Eligibility:

(Twenty lines not to exceed 75 characters per line)

1. Patients with poor prognosis ovarian cancer who have failed initial therapy or reinduction therapy including platinum analogues, and who have not been previously exposed to Taxol.
2. Age 18 to 60 years
3. Performance status 0-2 and acceptable cardiac, renal, hepatic, and pulmonary functions.

Treatment Plan: (Include dose adjustment)

(Twenty lines not to exceed 75 characters per line)

1. Patients with disease which is refractory to conventional dose combination chemotherapy will have marrow harvested following administration of conventional dose chemotherapy. The autologous marrow will be subjected to concentration in the COBE 2991 concentrator, CD34 selection in the CellPro Stem Cell Concentrator, and the cells modified with a safety-modified retrovirus which carries a CDNA for the multidrug resistance mRNA (MDR-1).
2. Autologous bone marrow infusion will take place following delivery of high dose cytoxan ($1.7 \text{ g/m}^2/\text{d}$) and thiotepa ($225 \text{ mg/m}^2/\text{d}$) each day for three days. Benadryl 25 mg and solucortef 100 mg will be given before bone marrow infusion to prevent anaphylaxis.
3. Patients will receive prophylactic oral antibiotics and blood products irradiated with 2500 rads.
4. Conventional dose Taxol starting at 135 mg/m^2 CI qdx1 each course, which will be given at increasing doses each course to tolerance given following recovery from transplant every three weeks. The patients will be treated for three courses at 135 mg/m^2 , then 200 mg/m^2 and then 250 mg/m^2 . The doses will continue to increase unless there is toxicity at unacceptable levels.

Patient Evaluation:

(Twenty lines not to exceed 75 characters per line)

	Pre-Treat.	Every 2-3 days	When WBC count > 1.5 K/ μ l every 3 months in remission
History, phys. exam	X		
CBC, differential and platelet counts	X	X	X
SMA12/PT/PTT/FIB/FSP and electrolytes*	X	X	X
BM aspirate & BX**	X		X
BM cytogenetics	X		
EKG/CXR/urinalysis*	X		

Pulmonary function test X

* In addition as indicated by clinical and hematologic situations

** Bone marrow aspirate and peripheral blood for PCR and functional evaluation of the MDR content of the cells at +21, +32 days post marrow reinfusion, and every three weeks during the first 6 months every 3 months during the first 2 years, and every 6 months for the first 5 years following transplant.

Miscellaneous Information:

(Include any other information that you feel is pertinent to the study)

(Three lines not to exceed 75 characters per line)

None

Statistical Considerations:

(Twelve lines not to exceed 75 characters per line)

We plan to initially study 15-20 advanced ovarian cancer patients in this pilot trial. We estimate that 100% of the patients will exhibit evidence of the functional MDR-1 sequences since chemotherapy selection in vivo will be used to select for the hematopoietic cells which have retained the MDR-1 retroviral transgenome.

Objectives:

(Twelve lines not to exceed 75 characters per line)

1. To evaluate the feasibility of introducing into early hematopoietic progenitor cells the MDR-1 cDNA in advanced ovarian cancer patients and thereby promoting the in vivo selection of genetically modified hematopoietic cells which are resistant to chemotherapy by virtue of having been transduced by the MDR-1 cDNA containing safety-modified retroviruses.
2. To determine the toxicity of modifying stem cells with MDR viruses on hematopoietic function following and during transplant.

OBJECTIVES

- 1.1 To test the feasibility of introducing the MDR-1 cDNA into normal hematopoietic early progenitor cells in advanced ovarian cancer patients through transduction of these cells with a safety-modified retrovirus which bears the MDR-1 cDNA in a transcription unit.
- 1.2 To determine the toxicity of modifying normal stem cells with MDR viruses on hematopoietic function following and during transplant.

BACKGROUND

Ovarian cancer strikes women in the prime of life. For those patients whose disease does not respond to regional approaches or conventional dose chemotherapeutic approaches to the control of the disease, an 80% mortality can be predicted. Furthermore, many of these patients exhibit signs of decreased marrow reserve at the time of attempted treatment of this residual disease, presumably due to the exposure of these patients to alkylating agents, irradiation, and other agents like Taxol, which on repetitive administration alone or in combination with other drugs, will result in a decrease in the rapidity of recovery following conventional dose chemotherapy.

The response of ovarian cancer cells exhibits dose dependence, suggesting that the delivery of intensive doses of chemotherapy would alter the unfavorable natural history of this disease. This in vitro data, and the failure of conventional dose combination chemotherapy to alter the poor prognosis of patients whose disease does not respond to initial therapy, led to the testing of the efficacy of intensive dose combination chemotherapy (1). Considerable interest in using intensive therapy supported by autologous transplants developed due to the fact that the marrow in ovarian cancer is almost never involved with cancer cells. The early trials with intensive therapy and autologous bone marrow transplantation did not lead to measurable increases in survival, although dramatic responses in terms of reductions in bulk disease were often seen (1-2). This suggested that the delivery of intensive but not ablative cyclical therapy following autologous bone marrow transplantation would lead to an improvement of survival. This expectation was based on the impressive responses of ovarian cancer cells to the intensive preparative regimens for autologous transplantation, and the persistence of small level disease during the post-transplant period. Unfortunately for these patients, the marrow graft is very fragile following transplantation such that it is not always feasible to deliver relatively intensive cyclical therapy following bone marrow transplantation.

During the early 70's, many investigators identified that epithelial neoplasms exhibited resistance to multiple drugs, and that if the cells were resistant to one member of this family of drugs, the tumor cells were often resistant to the other members of the family. Since the individual members of this family of drugs to which the tumor cells were cross resistant were not obviously related, Ling proposed

that a single protein, which was an efflux pump, conferred resistance to these drugs through generating a reduction in the intracellular concentrations of any one of these agents. Work designed to isolate this efflux pump protein culminated in the isolation of the p170 glycoprotein and the MDR-1 cDNA and gene.

Subsequent laboratory work on tumor cell lines which exhibited resistance to these drugs, or on tumor cell lines into which the cDNA for the MDR-1 cDNA was introduced by transduction, led to the conclusion that the MDR-1 cDNA conferred resistance to the following drugs: actinomycin D, VP-16, Velban, vincristine, adriamycin, and Taxol. Although there are multiple forms of the MDR cDNA both in mouse and man (MDR-1 and 3 in man and MDR 1-3 in the mouse), only one of the group in each species is functionally active in conferring resistance to these drugs (MDR-1 in man and MDR-3 in mouse). In addition, exposure of the cells of patients often leads to increases in the levels of the MDR-1 mRNA and in the protein GP170 for which it is the coding sequence.

Studies of patients with ovarian cancer have shown that the elevation or even the presence of detectable levels of the MDR-1 mRNA or protein in ovarian cancer cells is very uncommon (3-5), and that even after exposure to the MDR-1 drugs, that the levels of elevated MDR-1 (which are 2 to 3-fold higher than those which are detectable before treatment), are at least 3-fold lower than the levels which are achievable in hematopoietic stem cells which have been transfected with safety-modified retroviruses which carry a single MDR-1 cDNA in a transcription unit (6). In addition, animal model experiments in dogs, mice and in primates, have shown that it is feasible to modify the hematopoietic stem cells with safety-modified retroviruses which carry the MDR-1 cDNA in man or the MDR-3 cDNA in the mouse such that the hematopoietic cells following bone marrow transplant retain significant levels of cells which are genetically modified, and express the MDR-1 cDNA (5,7-8). Furthermore, these studies have shown that delivery of conventional dose chemotherapy to these animals results in increases in the levels of the MDR-1 cDNA and GP170 protein in the marrow and peripheral blood cells of these animals, and in the percentage of cells which carry the retroviral transgenome. In other words, these studies have shown that it is possible to increase the resistance of hematopoietic cells to the effects of MDR-1 drugs and that this increased resistance, which the MDR-1 retrovirus transduction confers upon the hematopoietic stem cells, results in the ability to select and maintain the genetically modified cells in the marrow of animals which have been transplanted with these modified cells by autologous bone marrow transplantation. No alteration in the maturation of hematopoietic cells has been seen in mice transplanted with MDR modified cells (8,14). Several groups have now shown that the presence of viral MDR-1 in transplanted marrow is resistant to Taxol and other MDR drugs (11-14).

We have proposed the use of peripheral blood and marrow CD34 positive cells for the transplant. Our institution has had experience with transplantation with the CD34 selected cells. We have seen recovery of the absolute neutrophil count to 500/cu mm in 10, 14, 14, and 17

days in breast cancer patients and 14, 17, 18, 18, 24, and 27 in advanced CML patients who have been transplanted with CD34 selected peripheral blood and marrow cells. The University of Denver has observed an average of 17 days to 500 neutrophils/cu mm in advanced breast cancer patients.

As shown in Figure 1, our own laboratory has shown that introduction of the MDR-1 cDNA containing safety modified retrovirus into the marrow cells of BALB-C mice before transplant shows that the marrow cells of the mice transplanted with the MDR-1 modified marrow are more resistant to Taxol than are mice transplanted with unmodified marrow.

The marrow become resistant to Taxol chemotherapy using transduction procedures which give integration of the virus into only 1-10% of the cells suggesting that in vivo selection of the MDR-1 modified cells occurs during chemotherapy. During the past two years, considerable progress has been made in the introduction of additional sequences into the somatic cells of patients in both constitutional as well as acquired molecular diseases (9-13). This has been accomplished with a family of safety-modified retroviruses which have been modified so that they cannot replicate in the normal or abnormal somatic cells of the patients. The sequences required for viral replication have been removed from the viruses, and placed in fragments in different parts of the genome of "producer cell lines", in which the replication incompetent safety-modified viruses can occur. These viruses are not harmful so long as they are not contaminated with replication competent helper virus, or viruses which can combine with the safety-modified viruses in a way which preserves the functional MDR-1 transgenome and restores (integrates) the rest of the replicating functions into the virus in an active array.

Producer cell lines can support the proliferation and replication of safety-modified viruses. The producer cell lines contain the replicative function of the virus. The safety-modified viruses can be grown in these cell lines. The use of safety-modified viruses from the cell lines has not led to the appearance of replication competent virus over the past two years in 250 laboratories. In addition, the use of these retroviruses in patients has not led to any abnormal disease state, or toxicity in patients in whom these viruses have been used for therapy (15). This data led to several therapeutic trials which involved the modification of the somatic cells of patients for genetic marking and for the replacement of missing or defective genes in a number of diseases. We have been involved in this work at M.D. Anderson, and have at the moment three protocols approved by the NIH Recombinant DNA Advisory Committee, and one protocol FDA-approved and ongoing at the M.D. Anderson Cancer Center at this time (16-19). We have shown in our own laboratory that we can achieve transduction frequencies in the 1-5% range even with the normal hematopoietic progenitor cells (17). Malcolm Brenner of St. Jude Children's Research Hospital has shown that these vectors, when used to modify the marrow of AML patients in remission, lead to a hematopoietic population following transplant which is positive for the retroviral transgenome at a frequency after transplant which equaled the

Analysis of Chemotherapy Induced Myelosuppression in BALB/c Mice

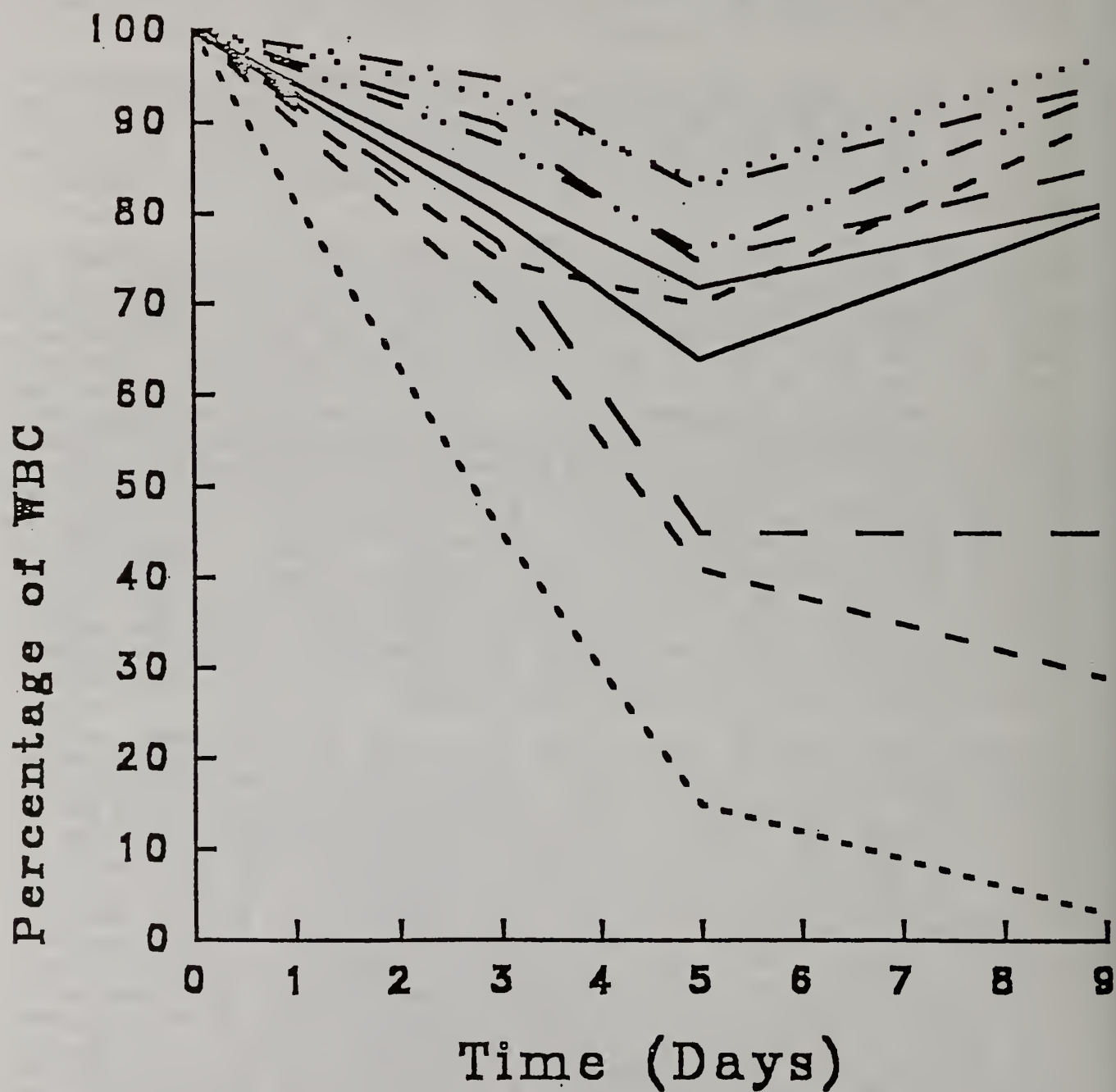


Figure 1

LEGEND FOR FIGURE 1

Mice were given chemotherapy following hematopoietic recovery (this requires 2-3 weeks). The chemotherapy was Taxol, freshly reconstituted at 7, 10, 14, 20, and 30 mg/kg.

The time course of recovery from Taxol-induced myelosuppression was measured following two or three doses of chemotherapy. The data from these experiments is shown in Figure 1 in which the following experimental groups are outlined:

Marrow containing Gottesman virus following:

- Curve 1: Two courses of 10 mg/kg Taxol;
- Curve 2: Three courses of 7 mg/kg Taxol;
- Curve 3: Three courses of 14 mg/kg Taxol;

Marrow containing our laboratory virus following:

- Curve 4: Three courses of 14 mg/kg Taxol;
- Curve 5: Three courses of 7 mg/kg Taxol;
- Curve 6: Two courses of 10 mg/kg Taxol;

Marrow containing no virus following:

- Curve 7: One course of 10 mg/kg Taxol;
- Curve 8: One course of 14 mg/kg Taxol;
- Curve 9: One course of 20 mg/kg Taxol;
- Curve 10: One course of 30 mg/kg Taxol;

The mice died following 20 and 30 mg/kg without virus.

This data shows a faster recovery and a reduction in the degree of myelosuppression following virus when given our laboratory virus and two or three courses of Taxol at 10 and 14 mg/kg. The degree of protection given by the Gottesman virus was similar to that observed with our own laboratory virus.

frequency of the marrow cells following in vitro exposure to the MDR viruses before transplant. These studies have also shown that the retroviruses is functional by the criterion of resistance to the bacterial resistance gene Neo. The presence of the retroviral transgenome in the cells has persisted up to 9-12 months. We have recently shown that we can achieve higher than 10% transduction frequency if we use stromal monolayers to support the growth of the hematopoietic cells during the transduction (19).

Interestingly, the studies of Brenner have been positive, the AML cells of these patients are also positive at the 10% level at the time of relapse, suggesting that all of the cells of marrow exposed to the safety-modified retroviruses are modified at the same level with the vector sequences. The data and our animal model data suggests that we will be able to modify the normal hematopoietic stem cells of these patients with the retroviruses which are safety-modified and contain a functional transcription unit with MDR-1 cDNA.

Accordingly, we have proposed to modify the marrow cells of refractory ovarian cancer patients, whose expectation of dying from the disease (by virtue of adverse prognostic features) is 80% or more. In this program, we will harvest the marrow following conventional dose chemotherapy, purify the early progenitor cells through CD34 selection, and then modify the marrow cells with a protocol outlined in Appendix D. We will then treat the systemic disease with a regimen which consists of high dose cyclophosphamide ($1.7 \text{ g/m}^2/\text{d} \times 3$) and thiotepa ($225 \text{ mg/m}^2/\text{d} \times 3$). This regimen was chosen as it has two alkylating agents which are active in ovarian cancer, and at the same time will provide suppression of the endogenous hematopoiesis needed to facilitate engraftment of the genetically-modified cells. These patients will be transplanted with the genetically modified stem cells. Following recovery of hematopoietic function, the patients will receive cyclical Taxol therapy with starting doses at 135 mg/m^2 . The dose will be increased by 50 mg/m^2 every course until toxicity is encountered. The courses will be delivered every three to four weeks, depending on recovering the ANC to $2000/\text{mm}^3$ and the platelet amount to $100,000/\text{mm}^3$. We will measure the levels of the cells which are modified by the MDR-1 viruses, and monitor the rate of hematopoietic recovery, which should become more rapid with each cycle and should contain higher and higher percentages of MDR-1 cDNA positive cells due to the selective effect of the chemotherapy. Finally, once this principle of in vivo selection is established, it will be possible to increase the doses of Taxol with each cycle of therapy. The goal of this program is to establish this principle of in vivo selection of genetically modified cells, and to study their effect on the ability to deliver very intensive doses of combination chemotherapy, or at least in the initial phases, relatively intensive doses of Taxol following recovery of hematopoietic function after autologous bone marrow transplantation. This may have a beneficial effect on the survival of these patients in whom the inability to eradicate residual disease lead to an 80% expectation of dying of their disease within two years.

3.0 BACKGROUND DRUG INFORMATION (SEE APPENDIX C)

PATIENT ELIGIBILITY

- 4.1 Advanced disease ovarian cancer patients who have not previously been exposed to Taxol and who have failed conventional dose therapy or induction therapy including platinum analogues and who therefore have an 80% chance of dying of progressive ovarian cancer.
- 4.2 Patients must have a performance of <3 on Zubrod performance scale (Appendix A1), a creatinine level <1.6 mg%, acceptable cardiac condition (class I or II; Appendix A2), normal liver functions with bilirubin <2 mg%, and acceptable pulmonary condition (FEV1 and DLCO $>50\%$ of predicted). Patients should be free of infections at the time of treatment.
- 4.3 A serum creatinine less than 1.5, a creatinine clearance of at least 50 cc/min/m², a SGOT within the normal range, and a bilirubin of less than 1.5 is required.
- 4.4 Patients with refractory disease must have an engrafting dose of a CD34 selection autologous bone marrow stored with the cells exposed to safety-modified retroviruses which contain the MDR-1 cDNA in a transcriptional unit. A backup marrow or peripheral blood stem cell collection which is equivalent to an engrafting dose must to be stored. Patients must sign informed consent, must not be pregnant or lactating, and must be practicing adequate contraception if of childbearing potential.
- 4.5 No patient who is unresponsive to platelet infusions will be eligible for this study unless a total of 8 collections of autologous platelets are available before therapy.

TREATMENT PLAN

- 5.1 General: All patients should be registered with the data management office, Ext: 2-2926. Information pertaining to important patient characteristics will be recorded.
- 5.2 Bone marrow aspiration and collection of peripheral blood stem cells and storage: Marrow is to be stored using in vitro methods for reducing the total number of mature nucleated cells and for the enrichment of the stored marrow in the early progenitor cells, by the CellPro Cephate Stem Cell Concentrator. The method of concentrating the marrow and for concentrating the marrow in early stem cells using the CellPro Cephate Collector are summarized in Appendix B.

The cells will then be modified by transduction with the safety-modified retroviruses which carry the MDR-1 cDNA in a transcription unit. The retroviruses are outlined in Appendix D. The method of transduction is outlined in Appendix D.

The criterion for adequacy of the autologous marrow will be as follows: At least 2×10^8 /kg nucleated cells per kg, 10,000 CFUGM/kg, 0.7×10^6 CD34 positive cells/kg, and a marking frequency of at least 1% in the marrow. If this is not available, an additional marrow storage will be conducted, or an equivalent number of progenitor cells, as measured by the CFUGM assay, will be collected from the peripheral blood and modified

as outlined in Appendixes B and D. A backup marrow or peripheral blood stem cell collection would be available which is genetically unmodified.

5.3 Treatment Plan: All patients should be treated in the Protected Environment if available.

- a. The preparative ablative regimen will consist of the following systemic chemotherapy:

Cyclophosphamide: 1.7 g/m^2 qd on days 1-3; mesna, 6 mg/kg will be given q6h during cyclophosphamide infusion.

Thiotepa at a dose of 225 mg/m^2 by continuous infusion over 4 days will be diluted in normal saline.

- b. Subsequently modified autologous bone marrow will be reinfused on day 9 after the last dose of chemotherapy following an interval of 6 days so as to protect the marrow from metabolites of the chemotherapy. Premedication with benadryl 25 mg and solucortef 100 mg ivb 30 minutes before reinfusion to prevent anaphylactic reactions.

- c. Treatment of patients in the Protected Environment on the 12th floor of the Lutheran Pavilion is strongly urged. Patients will remain there until the attainment of $500 \text{ granulocytes/mm}^3$.

- d. Patients will receive bactrim DS po BID and ketoconazole 200 mg po q8h as antibiotic prophylaxis or other antibiotics specified by the Infectious Disease group while hospitalized to protect patients against overwhelming infection.

- e. All blood products will be irradiated from the start of treatment and for three months following the transplant.

- f. GCSF (5 mcg/kg) will be given SC qd starting on day 1 after marrow harvesting and until the ANC is $\geq 10,000/\mu\text{d}$.

5.4 Maintenance Therapy: Taxol will be given every three to four weeks following recovery of hematopoietic function after bone marrow transplantation. The starting dose will be 135 mg/m^2 CI/24 hours, and will be given to 3 patients if hematologic, neurologic or mucocutaneous toxicity is ≤ 2 . Dose will be escalated to 200 mg/m^2 in the next cohort of 3 patients. If any of the patients develop grade 3 toxicity, 3 additional patients will be treated at the same dose level. If 2/6 patients develop grade 3 toxicity, it will be considered M.T.D. If there is no grade 3 toxicity, the dose will be further escalated by increments of 50 mg in cohorts of 3 patients until M.T.D. If the kinetics of hematopoietic recovery is such that increases in the doses of Taxol can be made, (a recovery of 2000 neutrophils/cm mm^3 , 100,000 platelets/cm mm^3 at day 21 after the previous course of therapy), hematologic recovery will be defined as an absolute neutrophil count (ANC) $> 2,000/\text{mm}^3$ and a platelet count $> 100,000/\text{mm}^3$.

Schema for Treatment Plan:

- I. Conventional dose chemotherapy.
- II. Collection of marrow to achieve a dose of 10,000 CFUGM/kg or a total nucleated count of 200 million nucleated cells/kg. The marrow will be concentrated using the 2991 COBE cell separator, and then separated using the CellPro Cephate Concentrator to enrich the preparation of stem cells in CD34 positive cells.
- III. Exposure of the engrafting dose of hematopoietic cells to a safety-modified retrovirus which contains a MDR-1 cDNA will be undertaken following the protocol outlined in Appendix D. PCR assay for the MDR cDNA will be performed following the transduction using the assay outlined in Appendix D. The PCR assay will be used to identify if the transduction was successful. A transduction frequency of 1% will be considered successful. Functional assays (rhodamine dye exclusion and resistance to Taxol in vitro, will also be used to monitor the success of the transduction. The marrow will be frozen away using programmed freezing. The viability of the frozen marrow will be tested using the CFUGM assay.
- IV. Preparative therapy will be delivered and will involve cyclophosphamide and thiotepa, in doses outlined earlier in this protocol.
- V. Infusion of autologous marrow and blood with PCR for MDR-1 of the thawed specimen before infusion.
- VI. PCR for MDR-1 and functional assays for MDR-1 on the hematopoietic cells following a recovery of an ANC of $2000/\text{mm}^3$ is achieved.
- VII. Delivery of relatively intensive but not ablative levels of therapy, at a starting dose of $135 \text{ mg}/\text{m}^2$ every three to four weeks, and the dose will be increased by $50 \text{ mg}/\text{m}^2$ increments each course as is allowed by the rate of hematopoietic recovery. PCR assays will be conducted to assess the percentage of hematopoietic cells which are positive for the MDR-1 cDNA after each course of chemotherapy.
- VIII PCR for MDR-1 on colonies of peripheral blood and marrow hematopoietic cells every three to four weeks following each course of therapy, as long as that therapy is administered, or until relapse, at which time the therapy of Taxol maintenance will no longer be administered. In situ functional analysis of the number of cells in peripheral blood and marrow by dye exclusion will be performed at the same time as PCR of MDR-1 to enumerate the number of cells with increased MDR-1 in the marrow and peripheral blood. After discontinuation of Taxol therapy, the PCR assays will be conducted every three months for two years, and every six months until a 5 year period has elapsed following the initial transplantation of modified marrow.

6.0 PRE-TREATMENT EVALUATION

- 6.1 A complete history and physical examination including documentation of all measurable disease, especially involvement of the peritoneum and other site of initial bulk disease which were established initially. A dental examination is also recommended.
- 6.2 Laboratory studies include CBC, platelet count, differential, SMA 12, liver and renal function tests, coagulation studies (PT, PTT, Fibrinogen, FSP), and cytogenetics. PCR for MDR-1 will be conducted as outlined above.
- 6.3 Bone marrow aspirate and biopsy for morphology, pathology, and cytogenetics.
- 6.4 An EKG and CXR will be performed on all patients. A urinalysis will also be obtained before therapy.
- 6.5 Pulmonary function studies with diffusion capacity.

7.0 EVALUATION DURING STUDY (SEE APPENDIX F)

- 7.1 CBC, platelet, differential every 1-2 days during the initial induction.
- 7.2 SMA 12, lytes every three to seven days and as indicated additionally.
- 7.3 Bone marrow aspirate and biopsy for morphologic pathology, cytogenetics, and PCR for MDR-1 should be performed at marrow recovery (when AML count $> 2000/\text{cm}^3$ mm).
- 7.4 Upon marrow recovery, a full work-up including CBC, platelet count and differential, SMA 12, and marrow studies including cytogenetics will be performed. Studies at remission will include CBC, platelet count, differential and SMA 12 every 1-4 weeks, marrow studies with cytogenetics every 6 months and as indicated by disease status.
- 7.5 The following will be collected before and after vector exposure and after hematopoietic recovery and on a six monthly basis for five years after transplant: 2×10^6 cells for MDR-1 PCR, 2×10^6 blood and marrow cells for viral PCR, 2×10^6 cells for NIH3T3 viral amplification studies, 1×10^7 cells for Southernns and 10 cc of a red top tube for Western blots for viral antibodies.

8.0 CRITERIA FOR RESPONSE AND TOXICITY (APPENDIX E)

- 8.1 Criteria for toxicity will be as follows: Failure of the marrow to engraft and the need to use back-up collections of hematopoietic cells from the marrow or peripheral blood for reconstitution.

- 8.2 Disordered hematopoiesis, abnormal morphology and alteration of the ratio of myeloid to erythroid cells will be established by morphology every three months as outlined in Appendix F.
- 8.3 Neurotoxicity or mucositis (Grade III) in 3 consecutive patients will be grounds for decreasing the Taxol dose.

9.0

CRITERIA FOR REMOVAL FROM STUDY

- 9.1 Patients whose marrow is not successfully marked with the MDR-1 retroviral vector, and whose marrow does not exhibit an increase in the level of resistance to the conventional dose chemotherapy with MDR drugs will not be continued on study unless there are preparations of marrow which contain elevated levels of hematopoietic cells which are resistant to MDR drugs above the levels which are encountered with unmodified cells.

10.0

STATISTICAL CONSIDERATIONS

- 10.1 For this pilot trial, evaluation of the persistence of the functional state of the MDR sequences introduced into marrow cells which are then transplanted will be conducted by aspiration of marrow cells following hematopoietic recovery from the transplant, and following each dose of post-transplantation therapy with Taxol for the first two years, or until the therapy is discontinued due to progression of disease, or until the death of the patient. PCR for MDR-1 will be used, and functional assays for the efficiency of efflux pumping by the marrow cells of dye rhodamine, or of the MDR drugs such as adriamycin, or by study of in vitro resistance of the modified cells. The PCR assay and functional assays of MDR-1 pumping will be done following the schedule outlined in Appendix F. The number of patients necessary for this program is 15-20. One patient per month will be evaluated and then study completed in one to two years.

The outcome which would lead to further study is that in in vitro and in vivo evidence of attenuating nadirs by 50% would prompt their investigators to further studies.

- 10.2 Evaluation of the response of the ovarian cancer to the therapy delivered as preparative therapy before bone marrow transplantation, and after the post-transplantation therapy of cyclical high dose Taxol, as outlined in the Treatment Plan.
- 10.3 Stopping Rules: If three successive patients have treatment deaths or failure to engraft, delayed hematopoietic recovery, or any treatment-related cause, the trial will be stopped. If the preparative therapy or the Taxol maintenance is ineffective in over 5 consecutive patients, the list will be stopped.

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17. Etkin, M., Filaccio, M., Ellerson, D., Suh, S.-P., Claxton, D., Gaozza, E., Brenner, M., Moen, R., Belmont, J., Moore, K., Moseley, A.M., Reading, C., Khouri, I., Talpaz, M., Kantarjian, H., and Deisseroth, A. *Human Gene Therapy* 3:137-145, 1992.
18. Deisseroth, A., Kantarjian, H., Talpaz, M., Champlin, R., Reading, C., Andersson, B., and Claxton, D. *Human Gene Therapy* 2:359-376, 1991.
19. Moore, K.A., Deisseroth, A.B., Reading, C.L., Williams, D.E., and Belmont, J.W. *Blood* 79:1393-1399, 1992.

The University of Texas
M.D. ANDERSON CANCER CENTER

INFORMED CONSENT

PROTOCOL TITLE: USE OF SAFETY-MODIFIED RETROVIRUSES TO INTRODUCE
CHEMOTHERAPY RESISTANCE SEQUENCES INTO NORMAL
HEMATOPOIETIC STEM CELLS FOR CHEMOPROTECTION
DURING THE THERAPY OF OVARIAN CANCER: A PILOT
TRIAL

1. _____
Participant's Name I.D. Number

You have the right to know about the procedures that are to be used in your participation in clinical research so as to afford you an opportunity to make the decision whether or not to undergo the procedure after knowing the risks and hazards involved. This disclosure is not meant to frighten or alarm you; it is simply an effort to make you better informed so you may give or withhold your consent to participate in clinical research. This informed consent does not supersede other informed consents you may have signed in other protocols.

DESCRIPTION OF RESEARCH

2. PURPOSE OF STUDY: This is a clinical research study with the following purpose: To make normal marrow cells resistant to chemotherapy and reduce the complications of therapy.
3. DESCRIPTION OF RESEARCH: In order to make normal marrow cells resistant to the side effects of chemotherapy, the following will be carried out: (A) The patient's own normal marrow and peripheral blood cells will be harvested from the peripheral blood using a pheresis machine and from the bone marrow by placing the needles into the hip bone during general anesthesia. These cells will then be made more resistant to chemotherapy by introducing a pumping mechanism, the MDR-1 gene, into the cell. This results in the pumping of the chemotherapy drugs from the inside to the outside of each cell, which protects it from therapy. We will insert the chemotherapy pump into the marrow cells by a carrier molecule which is taken from a virus. This is called a safety-modified virus. This molecule carries the genetic information for the membrane pump into the marrow cells, but does not cause an infection. (B) Two chemotherapy drugs will be given (cyclophosphamide and thiotepe) to eliminate the patient's marrow. The modified marrow cells which carry the chemotherapy pump gene will then be transfused. This will be given by vein in a manner which is similar to any blood transfusion. The

recovery of full marrow function (with normal blood counts) will require 3-4 weeks in the hospital. During this time, IV fluids and antibiotics may be necessary. (C) Following recovery of the modified marrow, a chemotherapy drug which is affected by the chemotherapy pump, Taxol, will be given by intravenous infusion. Taxol is a drug which is very effective against ovarian cancer, particularly at the late stages. This will be administered every three to four weeks depending on how rapidly the white blood cell counts recover after each infusion. The marrow cells will be protected from the Taxol by the chemotherapy pump, but the tumor cells which do not contain the pump, will die. (D) The patients participating on this trial will be followed for 5 years in remission or until relapse. 15-20 participants will be entered in this trial. In case of death, the patient's family will be asked for an autopsy.

4. RISKS, SIDE EFFECTS AND DISCOMFORTS TO PARTICIPANTS:

The side effects of the cyclophosphamide and thiotepa include fever, loss of hair, nausea and vomiting, infections, mouth sores, reversible cardiac failure, diarrhea and bladder inflammation and bleeding. The side effects of Taxol include suppression of marrow function and neurological changes. The treatment related mortality of the cyclophosphamide and thiotepa is estimated to be less than 5%, since no patient died receiving this regimen, and the dose of one of the drugs (cyclophosphamide) has been further reduced. It is possible that the toxicity of the Taxol could be increased immediately following the transplant, but the dose of the Taxol has been reduced to prevent this toxicity.

Extensive study of the use of safety-modified viruses to introduce new information into cells has already been accomplished in animal models (mice, dogs, and small subhuman primates). This virus and another like it are currently being studied at other institutions. No toxic effects have been observed in all of this study because the virus is modified so that it cannot cause an infection in the cells of the body. It only inserts new information into a small number of blood cells in the marrow or blood.

Risks, however, which may not yet have been observed, are possible. These are "theoretical risks" since they have not yet been seen. It is possible that the virus could change the cells which are marked so as to grow in an abnormal pattern, and even cause cancer or leukemia. This risk is much lower than damage to the cells by chemotherapy and irradiation. The marker might also produce a protein which might inactivate certain antibiotics but alternative antibiotics are available so that this does not constitute a risk or threat for patients during therapy.

- 4a. This clinical research may involve unforeseeable risks to the participant (or to the embryo or fetus, if the participant is or may become pregnant during treatment). To help prevent injury to unborn children, upon recommendation by the attending physician, the participants should practice adequate methods of birth control throughout the period of their involvement in this clinical research study.
5. **POTENTIAL BENEFITS:** The use of chemotherapy resistant cells will potentially allow the use of higher doses of chemotherapy, therefore increasing the chances to destroy the ovarian cancer cells.
6. **ALTERNATE PROCEDURES OR TREATMENTS:** Participation in this marking study is totally optional and other treatment options for this disease exist although none are known to alter the course of this disease at this time and at this stage of the disease. These include conventional dose chemotherapy without modified cells.

UNDERSTANDING OF PARTICIPANTS

7. I have been given an opportunity to ask any questions concerning the investigational drug regimen involved and the investigator has been willing to reply to my inquiries. This investigational drug regimen will be administered under the above numbered, titled and described clinical research protocol at this institution. I hereby authorize Dr. _____, the attending physician/investigator and designated associates to administer this investigational drug regimen.
8. I have been told and understand that my participation is voluntary. I may decide not to participate, or withdraw my consent and discontinue my participation at any time. Such action will be without prejudice and there shall be no penalty or loss of benefits to which I may otherwise be entitled, and I will continue to receive treatment by my physician at this institution.

Should I decide not to participate or withdraw my consent from participation in this clinical research, I have been advised that I should discuss the consequences or effects of my decision with my physician.

In addition, I understand that the investigator may discontinue the clinical research study if, in the sole opinion and discretion of the investigator, the study or treatment offers me little or no future benefit, or the supply of medication ceases to be available or other causes prevent continuation of the clinical research study. The investigator will notify me should such circumstances arise and my

physician will advise me about available treatment which may be of benefit at that time.

I will be informed of any new findings developed during the course of this clinical research study, which may relate to my willingness to continue participation in the study.

9. I have been assured that confidentiality will be preserved except that qualified monitors from the Food and Drug Administration and the National Cancer Institute may review my records where appropriate and necessary. Qualified monitors shall include assignees authorized by the Surveillance Committee of this institution provided that confidentiality is assured and preserved. My name will not be revealed in any reports or publications resulting from this study, without my expressed consent.
10. I have been informed that should I suffer any injury as a result of participation in this research activity, reasonable medical facilities are available at this institution. I understand, however, that I cannot expect to receive any credit or reimbursement for expenses from this institution or any financial compensation from this institution for such injury.
11. I have been informed that I should inquire of the attending physician whether or not there are any services, investigational agents or devices, and/or medications being offered by the sponsor of the clinical research project at a reduced cost or without cost. Should the investigational agent become commercially available during the course of the study, I understand that I may be required to cover the cost of subsequent doses.

Costs related to my medical care including expensive tests or procedures that may be specifically required by this clinical research study shall not be my responsibility. I have been given the opportunity to discuss the expenses or costs associated with my participation in this research activity.
13. I understand that practicing effective contraception is medically necessary and a prerequisite for my participation in this clinical research study. Should contraception be interrupted or if there is any suspicion of pregnancy, my participation in this clinical research study will be terminated at the sole discretion of the investigator.
14. I may discuss questions or problems during or after this study with Dr. Albert Deisseroth at (713) 792-8750. In addition, I may discuss any problems I may have or any questions regarding my rights during or after this study with the Chairman of the Surveillance Committee at (713) 792-3220 and may in the event any problem arises during this clinical research contact the parties named above.

CONSENT

Based on the above, I consent to participate in the research and have received a copy of the consent form.

DATE

SIGNATURE OF PARTICIPANT

WITNESS OTHER THAN PHYSICIAN
OR INVESTIGATOR

SIGNATURE OF PERSON RESPONSIBLE
AND RELATIONSHIP

I have discussed this clinical research study with the participant and/or his or her authorized representative, using a language which is understandable and appropriate. I believe that I have fully informed this participant of the nature of this study and its possible benefits and risks, and I believe that the participant understood this explanation.

PHYSICIAN/INVESTIGATOR

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME	POSITION TITLE
Albert B. Deisseroth, M.D., Ph.D.	Professor and Chairman

EDUCATION(*Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.*)

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
Univ. of Rochester, Rochester, NY	A.B.	1963	Chemistry
Univ. of Rochester School of Medicine, NY	Ph.D.	1968	Biochemistry
Univ. of Rochester School of Medicine, NY	M.D.	1970	Medicine
Intern & Junior Resident, Beth Israel Hospital, MA	Resident	1970-1972	Internal Medicine
Sidney Farber Center	Fellow	1975-1976	Hematology/Oncology

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

POSITIONS HELD

1970 - 1972	Intern and Resident, Beth Israel Hospital, Boston, MA
1972 - 1974	Clinical Associate, NHLI, NIH
1974 - 1975	Chief Resident Physician, NHLI, NIH
1975 - 1976	Clinical Fellow, Hematology/Oncology, Children's Hospital Medical Center, Boston, MA
1976 - 1981	Head, Experimental Hematology Section, Pediatric Oncology Branch, NCI, NIH
1981 - 1987	Chief, Hematology/Oncology Section, Department of Medicine, UCSF Service, San Francisco VA Medical Center, and Professor of Medicine, UCSF
1987 - present	Prof. of Medicine and Chairman, Dept. of Hematology, U.T. M.D. Anderson Cancer Center, Houston, TX
1987 - present	Anderson Professor of Cancer Treatment and Research, M.D. Anderson Cancer Center, Houston, TX

RELEVANT PUBLICATIONS: (Selected from 186 publications)

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BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME Richard E. Champlin, M.D.		POSITION TITLE Professor of Medicine	
EDUCATION (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)			
INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
Purdue University, Indiana	B.S.	1971	Engineering Science
University of Chicago, Illinois	M.D.	1975	Medicine

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

HONORS: Graduated with distinction - Purdue University, 1971
 Graduated with honors - University of Chicago School of Medicine, 1975
 Alpha-Omega-Alpha Honorary Fraternity - University of Chicago, 1975

TRAINING:
 1975-1978 UCLA Harbor General Hospital, Internship and Residency in Medicine
 1978-1980 UCLA Center for the Health Sciences
 Fellowship in Hematology and Oncology

MEDICAL LICENSES: States of Texas and California, DEA AC7057006

FACULTY APPOINTMENTS:
 1981-1985 Assistant Professor of Medicine, UCLA School of Medicine
 1985-1990 Associate Professor of Medicine, UCLA School of Medicine
 1983-1990 Director, Transplantation Biology Program
 UCLA Johnsson Comprehensive Cancer Center
 1985-1990 Director, UCLA Hematology/Oncology Fellowship Program
 1990 Professor of Medicine, University of Texas, M.D. Anderson Cancer Center
 Chief, Section of Bone Marrow Transplantation
 1991 Consulting Staff, The Methodist Hospital, Internal Medicine and Hematology/Oncology

SOCIETY MEMBERSHIPS:
 American Association for the Advancement of Science
 American Association for Cancer Research
 American College of Physicians, Fellow
 American Federation of Clinical Research
 American Society of Hematology
 International Society for Experimental Hematology
 Western Society for Clinical Investigation

BOARD CERTIFICATIONS:
 American Board of Internal Medicine
 Internal Medicine 1978, Hematology 1980, Oncology 1981

PUBLICATIONS:
 1. CHAMPLIN RE, Gale RP. Acute myelogenous leukemia: recent advances in therapy. Blood 1987;69:1551-1562.

2. CHAMPLIN RE, Gale RP. The role of bone marrow transplantation in the treatment of hematologic malignancies and solid tumors: a critical review of syngeneic, autologous and allogeneic transplants. *Cancer Treat Rep.* 1984;68:145-161.
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Give the following information for the key personnel and consultants listed on page 2. Begin with the Principal Investigator/Program Director. Photocopy this page for each person.

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- 1988-89 Bristol Laboratories, Phase II Study of Carboplatin vs VP16 in Ovarian Cancer. \$72,000
- 1988-89 Basic Science Research Grant, Loss of Heterozygosity Studies in Ovarian Cancer, M.D. Anderson Cancer Center, Houston, TX. \$10,000
- 1989 Lilly Research, Phase II Study of LY186641 (Sulfonylurea) In Ovarian Cancer. \$65,000

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HUMAN GENE TRANSFER PROTOCOL

9306-045 Nabel, Gary J., University of Michigan Medical Center, Ann Arbor, Michigan; *Immunotherapy for Cancer by Direct Gene Transfer into Tumors.*

Date of RAC Approval: 6/7/1993

Date of NIH Approval: 9/3/1993

16.0 Scientific Abstract

The goal of immunotherapy is to stimulate the immune system by modification of tumor cells or expansion of lymphocytes which respond specifically to tumor antigens. In this study, we will apply techniques of direct gene transfer to enhance immune response against tumors *in vivo*. Patients with advanced cancer who have failed all effective therapy will be treated by injection of a DNA/liposome complex directly within the tumor. DNA will be used which encodes a heterodimeric cell surface protein recognized in the transplantation response. These genes include the HLA-B7 histocompatibility antigen and β -2 microglobulin gene in a non-viral plasmid eukaryotic expression vector. For this vector, a safe and effective dose to introduce this recombinant gene in HLA-B7⁺ patients will be established. HLA-B7 expression will be confirmed *in vivo*, and the immune response stimulated by the expression of this antigen will be characterized. We will also determine whether this treatment facilitates tumor regression alone or in combination with other treatment modalities. This study will employ a similar strategy to our previous gene therapy protocol, but employs four improvements in technology, including more efficacious liposomes, optimize vector expression, catheter delivery and application to other several types of cancer. These studies will facilitate the development of other approaches, using different recombinant genes or in combination with cytokines or adoptive T cell therapy, to augment tumor immunity, and allow for greater potential efficacy. This method will also establish the safety

on this non-viral approach to gene therapy, which could potentially be extended to treat a variety of other human diseases.

17.0 Non-technical Abstract

Many types of cancer cannot be cured by traditional medical treatments, including drugs, surgery, or radiation. In this study, an experimental treatment will be offered that may help to fight this disease. We will attempt to induce tumor regression by the introduction of genetic material that directs the synthesis of proteins which stimulate the immune system. The genetic material, DNA, will be introduced directly into the tumor by mixing it with fat bodies, or liposomes, and this mixture will be injected into the tumor or delivered by a catheter. The DNA will be taken into cells and cause them to produce proteins that stimulates tissue rejection. These proteins, called histocompatibility proteins, cause cells which contain it to be recognized as foreign by the immune system. In some cases, another immune stimulatory protein, interleukin, will also be included. The goal of the treatment is to stimulate the immune system to attack and kill the tumor. In this study, we will determine a safe and effective dose to administer the DNA liposome complex. Increasing amounts of this complex will be used in different patient populations. If no side effects are observed, repeated treatments will be instituted. The expression and nature of the immune response will also be characterized. This treatment may provide a therapeutic effect in cancer and could be applied to the treatment of other diseases.

"Immunotherapy for Cancer by Direct Gene Transfer into Tumors"

HUMAN GENE THERAPY PROTOCOL

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1.0 Introduction

1.1 Rationale and Relationship to Previous Work

This proposal represents an extension of a previously approved protocol submitted and approved by the Recombinant DNA Advisory Committee on February 10, 1992, entitled "Immunotherapy of Malignancy by *In Vivo* Gene Transfer into Tumors." Since the implementation of the first protocol, there have been four developments which have led to the modifications proposed in this study. The background and description of the previous trial is included in the appendix (1-5). The previous trial has now been completed, and the conclusions have been summarized in the accompanying data. Briefly, the trial has demonstrated that recombinant gene expression can be achieved following direct gene transfer into melanoma in humans. In addition, there has been no evidence of toxicity from this treatment, and no antibody responses were detected to DNA as a result of this intervention. The clinical and immunologic responses are currently undergoing evaluation, and the status of these studies will be summarized at the RAC meeting; however, based on the findings of gene expression and lack of toxicity, we have felt that it is appropriate to pursue this general approach to the introduction of a foreign antigen into tumors in an effort to stimulate anti-tumor immunity.

Since the initial trial, there have been advances in four areas of the gene transfer technology. In this protocol, we will incorporate these improvements into the protocol. Briefly, these improvements include:

1) development of a more efficacious cationic liposome which improves efficiency of gene delivery

2) improvements in vector design which further enhance expression *in vivo*

3) inclusion of catheter-based gene delivery

4) application to different tumor cell types

The preliminary data regarding each of these developments is shown in Section 13 (Preliminary Data). Briefly, a new formulation of cationic lipids has been described recently by Dr. Phillip Felgner in which a different cationic lipid, 1,2-dimyristyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide (DMRIE), is utilized with dioleoyl phosphatidylethanolamine (DOPE). This has two properties which make it more suitable for these studies. First, it shows up to 10-fold improved transfection efficiency compared to our previous formulation, DC-chol, *in vitro*. More importantly, this formulation does not aggregate at high concentrations, in contrast to the DC-Chol liposome. This characteristic thus allows higher absolute concentrations of DNA and liposomes to be introduced into experimental animals without toxicity (Section 13, Preliminary Data). Because of these properties, it now becomes possible to introduce 100-1000 times more DNA which could markedly improve gene expression *in vivo* (Section 13, Preliminary Data).

The vector improvements are divided into two categories for this proposal. In the first case, expression of the HLA-B7 vector has been improved by the addition of a consensus translation initiation sequence and removal of an intron. In

addition, the inclusion of the β -2 microglobulin gene, with which class I MHC genes normally associate, allows synthesis of the complete histocompatibility molecule, which is composed of these two chains. Ordinarily, these two gene products are co-transported to the cell surface, and some human melanoma cells do not express endogenous β -2 microglobulin, thus limiting their ability to stably express class I on the cell surface. We have found that the inclusion of the β -2 microglobulin gene on the same plasmid allows for the expression in these otherwise resistant cells and improve expression in other cells, thus overcoming a potential mechanism of resistance. A potential future modification of the vector involves the expression of a cytokine gene in addition to class I MHC and β -2 microglobulin. The elaboration of cytokines such as IL-2 or GM-CSF could further stimulate T cells immunity against the tumor locally and improved recognition of tumor-associated antigens. In experimental animal models, the introduction of IL-2 has allowed for improved anti-tumor efficacy ((6); see Section 13, Preliminary Data). To maximize the safety concerns for vectors which produce cytokines in the future, we would include the cytokine gene on the same transcript as the class I MHC gene, linking the expression of the cytokine gene to expression of the foreign histocompatibility antigen. In this way, cells which contain the cytokine gene would be eliminated after several weeks *in vivo*, minimizing any concerns regarding persistent expression of cytokines *in vivo*. At present, such a vector is not yet available; however, we would

ask to include it if it proves more effective than previous vectors in the future.

The third amendment to the protocol is the delivery of recombinant genes using a catheter. This modification was proposed in our previous trial as a minor amendment for one patient in the protocol with a pulmonary metastases. This treatment was well-tolerated by the patient. No complications or toxicities were noted (summarized in Section 13, Preliminary Data, Table 3). In this trial, we propose to analyze the toxicity associated with catheter-based gene delivery separately, since the toxicities of this procedure may differ from direct intratumor injection. This intervention provides the ability to transduce a larger percentage of cells within the tumor microcirculation in order to achieve greater efficacy of gene expression, at the same time minimizing the potential for inadvertent microscopic seeding of tumor cells to distant sites. Because this approach is not always feasible, we have also elected to continue the direct intratumoral injection approach. Finally, we have shown previously that this method of direct gene transfer is applicable to different tumor cell types. Particularly, for the *in vivo* catheter delivery, it may be appropriate to utilize this approach on malignancies other than melanoma. We therefore would like to maintain the option to treat other human cancers, for example, colon carcinoma, renal cell carcinoma, or breast cancer, in this study. These cancers would most likely be treated by catheter delivery.

Because of these several improvements, we have submitted a protocol which proposes to incorporate these modifications in another phase I/II clinical trial to determine the safety and toxicity of this treatment. The study design and clinical endpoints remain almost identical to the previous trial, and are detailed in the following sections.

1.2 Background

A variety of genetic abnormalities arise in human cancer which contribute to neoplastic transformation and malignancy. Instability of the genome generates mutations which alter cell proliferation, angiogenesis, metastasis, and tumor immunogenicity. Despite a better understanding of the molecular basis of cancer, many malignancies remain resistant to traditional forms of treatment. The definition of tumor-associated genetic mutations, however, has heightened interest in cancer as a target for gene therapy. Immunotherapy has shown promise as a primary approach to the treatment of malignancy. Indeed, specific cancers, such as melanoma or renal cell carcinoma, are relatively more responsive to modulation of immune function, possibly because the immune system can be induced to recognize mutant gene products in these cells. Conventionally, approaches to immunotherapy have involved the administration of non-specific immunomodulating agents such as *Bacillus Calmette-Guerin* (BCG), cytokines, and/or adoptive T cell transfer, which have shown promise in animal models (7-12) and in man (13-16). More recently, molecular genetic interventions have been designed in an attempt to improve the efficacy of immunotherapy. Human

gene transfer protocols have been designed to monitor the traffic of lymphocytes into melanoma tumors (17) or to introduce cytokine genes into tumor cells to stimulate the host's immune response to residual tumor (18).

We have recently developed a novel molecular genetic intervention for human malignancy. This approach relies on the direct transmission of recombinant genes into established tumors *in vivo* to genetically modify them as they grow *in situ*. In animal models, introduction of a gene encoding a foreign major histocompatibility (MHC) protein (class I) *in vivo* signals the immune system to respond to the foreign antigen (5,19). More importantly, when this gene is transduced into established tumors *in vivo*, a cytolytic T cell response is also generated against unmodified tumor cells. In murine models, this approach has led to significant reductions in tumor growth and, in some cases, complete remission (5). Based on these studies, we received approval from the Recombinant DNA Advisory Committee of the National Institutes of Health to conduct a human clinical protocol using direct transfer of a human transplantation antigen gene in an effort to treat malignancy. This protocol proposed to perform direct gene transfer in humans and to utilize a non-viral vector which reduces several safety concerns about viral vectors. This clinical trial involved the treatment of patients with metastatic melanoma at subcutaneous lesions. The treatment consists of intratumoral injection of the human class I MHC gene, HLA-B7 complex, to a cationic liposome, DC-Cholesterol (2,20). These patients received escalating doses of the DNA liposome

complex. Recombinant gene expression, toxicity, and the immunologic response to treatment is being evaluated. The objectives of this first human clinical trial using direct gene transfer were (1) to establish a safe and effective dose to introduce recombinant genes *in vivo*, (2) to confirm expression of the transduced gene, and (3) to analyze the immune response and potential therapeutic effects of this method of therapy. Based on animal studies, no toxicities had been readily apparent using these modes of direct gene transfer *in vivo* in short-term or long-term studies (1-3). Taken together, these studies were intended to determine whether direct gene transfer was an appropriate form of treatment for malignancy. In addition, the data compiled from this study also provided an indication of whether this method of delivery could be appropriate for the treatment of a variety of other human diseases.

In this study, another phase I clinical trial is proposed. The safety of this method and appropriate dosage in humans will be tested. Recombinant gene expression *in vivo* will be confirmed, and the specificity and mechanism of immune rejection will be defined. In subsequent phases, this response will be augmented by preimmunization and administration of cytokines, including tumor necrosis factor- α , interferon- γ , or interleukin-2, or used in combination with adoptive transfer or TIL therapy. These studies will provide an alternative strategy for the immunotherapy of malignancy and allow definition of the mechanism of immune rejection of tumor cells. Adaptations of this method may also eventually be applied to the treatment of other human

diseases.

1.3 Direct Gene Transfer and Modulation of the Immune System

The utilization of catheter-based gene delivery *in vivo* provided a model system for the introduction of recombinant gene-specific sites *in vivo*. Early studies focused on the demonstration that specific reporter genes could be expressed *in vivo* (21,22). Subsequent studies were designed to determine whether specific biologic responses could be induced at sites of recombinant gene transfer. To address this question, a highly immunogenic molecule, a foreign major histocompatibility complex (MHC), was used to elicit an immune response in the iliofemoral artery using a porcine model. The human HLA-B7 gene was introduced using direct gene transfer with a retroviral vector or DNA liposome complex (19). With either delivery system, expression of the recombinant HLA-B7 gene product could be demonstrated at specific sites within the vessel wall. More importantly, the expression of this foreign histocompatibility antigen induced an immunologic response at the sites of genetic modification. This response included a granulomatous mononuclear cell infiltrate beginning 10 days after introduction of the recombinant gene. This response resolved by 75 days after gene transfer; however, a specific cytolytic T cell response against the HLA-B7 molecule was persistent. This study demonstrated that a specific immunologic response could be induced by the introduction of a foreign recombinant gene at a specific site *in vivo*. Moreover, this provided one of the first indications that

direct gene transfer of specific recombinant genes could elicit an immune response to the product of that gene *in vivo* (19).

These studies suggested that the introduction of the appropriate recombinant genes could be used to stimulate the immune system to recognize its product *in vivo*. In addition, this approach provided a general method for the induction of a specific site *in vivo*. To determine whether direct gene transfer might be appropriate for the treatment of disease, a murine model of malignancy has been developed. Direct gene transfer of an allogeneic histocompatibility complex gene into a murine tumor elicits an immune response not only to the foreign MHC gene but also to previously unrecognized tumor-associated antigens. These immune responses are T cell-dependent, and these tumor-associated proteins are recognized within the context of the self major histocompatibility complex. In animals presensitized to a specific MHC haplotype, direct gene transfer into established tumors could attenuate tumor growth or, in some cases, lead to complete tumor regression (5). These studies demonstrate that direct gene transfer of foreign MHC genes into tumors have potentially therapeutic effects that may be appropriate for the treatment of malignancy.

1.4 Immunotherapy of Malignancy.

In some instances, the immune system appears to contribute to the surveillance and destruction of neoplastic cells, either by mobilization of cellular and humoral immune effectors. Cellular mediators of anti-tumor activity include MHC-restricted cytotoxic T cells, natural killer (NK) cells (23,24) and

lymphokine-activated killer (LAK) cells (25). Cytolytic T cells which infiltrate tumors have been isolated and characterized (26). These tumor infiltrating lymphocytes (TIL) selectively lyse cells of the tumor from which they were derived (10,27). Macrophages can also kill neoplastic cells through antibody-dependent mechanisms (28,29), or by activation induced by substances such as BCG (30).

Cytokines can also participate in the anti-tumor response, either by a direct action on cell growth or by activating cellular immunity. The cytostatic effects of tumor necrosis factor- α (TNF- α) (31) and lymphotoxin (32) can result in neoplastic cell death. Interferon- γ (IFN- γ) markedly increases class I MHC cell surface expression (33,34) and synergizes with TNF- α in producing this effect (35). Colony stimulating factors such as G-CSF and GM-CSF activate neutrophils and macrophages to lyse tumor cells directly (36), and interleukin-2 (IL-2) activates Leu-19+ NK cells to generate lymphokine activated killer cells (LAK) capable of lysing autologous, syngeneic or allogeneic tumor cells but not normal cells (25,37,38). The LAK cells lyse tumor cells without preimmunization or MHC restriction (39). Interleukin-4 (IL-4) also generates LAK cells and acts synergistically with IL-2 in the generation of tumor specific killer cells (40).

Since most malignancies arise in immunocompetent hosts, it is likely that tumor cells have evolved mechanism to escape host defenses, perhaps through evolution of successively less immunogenic clones (41). Deficient expression of class I MHC

molecules limits the ability of tumor cells to present antigens to cytotoxic T cells. Freshly isolated cells from naturally occurring tumors frequently lack class I MHC antigen completely or show decreased expression (42-46). Reduced class I MHC expression could also facilitate growth of these tumors when transplanted into syngeneic recipients. Several tumor cell lines which exhibit low levels of class I MHC proteins become less oncogenic when expression vectors encoding the relevant class I MHC antigen are introduced into them (47-51). In some experiments, tumor cells which express a class I MHC gene confer immunity in naive recipients against the parental tumor (48,49). The absolute level of class I MHC expression however, is not the only factor which influences the tumorigenicity or immunogenicity of tumor cells. In one study, mouse mammary adenocarcinoma cells, treated with 5-azacytidine and selected for elevated levels of class I MHC expression did not display altered tumorigenicity compared to the parent line (52).

The immune response to tumor cells can be stimulated by systemic administration of IL-2 (53), or IL-2 with LAK cells (54,55). Clinical trials using tumor infiltrating lymphocytes are also in progress (17). Recently, several studies have examined the tumor suppressive effect of lymphokine production by genetically altered tumor cells. The introduction of tumor cells transfected with an IL-2 expression vector into syngeneic mice stimulated an MHC class I restricted cytolytic T lymphocyte response which protected against subsequent rechallenge with the parental tumor cell line (6). Expression of IL-4 by plasmacytoma

or mammary adenocarcinoma cells induced a potent anti-tumor effect mediated by infiltration of eosinophils and macrophages (56). These studies demonstrate that cytokines, expressed at high local concentrations, are effective anti-tumor agents.

We have previously proposed an alternative approach to stimulate an anti-tumor response, through the introduction of an allogeneic class I MHC gene into established human tumors. The antigenicity of tumor cells has been altered previously by the expression of viral antigens through infection of tumor cells (57-61), or expression of allogeneic antigens introduced by somatic cell hybridization (62,63). Allogeneic class I MHC genes have been introduced into tumor cells by transfection and subsequent selection *in vitro*. These experiments have produced some conflicting results. In one case, transfection of an allogeneic class I MHC gene (H-2L^d) into an H-2^b tumor resulted in immunologic rejection of the transduced cells and also produced transplantation resistance against the parent tumor cells (64). In another instance, transfection of H-2^b melanoma cells with the H-2D^d gene did not lead to rejection (65), however increased differential expression of H-2D products relative to H-2K may have affected the metastatic potential and immunogenicity of tumor cells (66). The effects of allogeneic H-2K gene expression in tumor cells was examined in another study (67). Several subclones which were selected *in vitro* and expressed an allogeneic gene were rejected in mice syngeneic for the parental tumor line, however, other subclones did not differ from the parental, untransduced line in generating tumors. This finding

suggests that clone-to-clone variation in *in vivo* growth and tumorigenic capacity may result in other modifications of cells, caused by transfection or the subcloning procedure, which affects their tumorigenicity. These types of clonal differences would likely be minimized by transducing a population of cells directly *in vivo*.

Because the H-2K class I MHC antigen is strongly expressed on most tissues and can mediate an allogeneic rejection response, we chose it in our animal model studies designed to enhance the immunogenicity of tumors *in vivo*. These studies extended previous efforts to modify tumor cells by developing a system for the direct introduction of genes into tumors by *in vivo* infection using retroviral vectors or by DNA/liposome mediated transfection. This technology can also be used to deliver specific recombinant cytokines into the tumor microcirculation and to understand the immunologic basis for tumor rejection *in vivo*.

2.0 Objectives

The immune system can provide protection against cancer and may play an important role as an adjuvant treatment for malignancy. Lymphokine activated killer cells (LAK) and tumor infiltrating lymphocytes (TIL) can lyse neoplastic cells and produce partial or complete tumor rejection. Expression of cytokine genes in malignant cells has also enhanced tumor regression. Because current strategies to stimulate an immune response against tumor cells often fail to eradicate tumors, an important goal of immunotherapy is to improve upon current techniques and understand the mechanisms of immune recognition.

In this study, we attempt to improve upon our previous studies to enhance the immune response against tumors. We have utilized direct gene transfer into tumor cells *in vivo* as a method to simplify the delivery of genes *in vivo*. Traditionally, gene transfer techniques have focused on modification of tumor cells *in vitro*, followed by transfer of modified cells. Such approaches subject these cells to selection and different growth conditions from those which act *in vivo*. Because they also require that cell lines be established for each malignancy, adaptability to human disease is more difficult and requires more time.

We have previously described a model for the immunotherapy of malignancy using a gene encoding a transplantation antigen, an allogeneic class I major histocompatibility complex (MHC) antigen, introduced into human tumors *in vivo* by DNA/liposome transfection (1,2). Expression of allogeneic MHC antigens on

tumor cells stimulates immunity against both the allogeneic MHC gene on transduced cells as well as previously unrecognized antigens in unmodified tumor cells (5). The introduction of an allogeneic MHC gene directly into tumors *in vivo* has induced partial tumor regressions, as well as the specific cytotoxic T cell response to other antigens. In a recent trial in humans, we observed no toxicity of this form of treatment. We have continued to optimize this gene delivery approach, and now propose four improvements on the previous protocol which could improve its ultimate efficacy. These modifications include 1) the use of another cationic lipid formulation, DMRIE/DOPE, 2) optimization of vector expression and inclusion of a cytokine gene, 3) catheter-mediated gene delivery, and 4) application to different tumor types.

Because this approach employs direct gene transfer *in vivo*, it can be applied easily in a clinical setting to spontaneously arising tumors, alone or in combination with cytokines or other adjuvant treatments, including adoptive lymphocyte transfer, to augment tumor immunity. In this study, we propose another phase I clinical trial almost identical to our previous protocol which will evaluate the safety and appropriate dosage for these new modifications. Recombinant gene expression *in vivo* will be documented, and the specificity and mechanism of the immune response will be characterized. Escalating treatment regimens will be used and tumor growth evaluated. These studies will define the safety of the modifications of this approach to the immunotherapy of malignancy and may provide therapeutic effects

for patients. Adaptations of this approach might also prove useful in the treatment of other human diseases.

Specifically, we plan:

- 1) To establish a safe and effective dose to introduce either a plasmid vector encoding recombinant HLA-B7 with β -2 microglobulin. Potentially, a plasmid vector encoding recombinant HLA-B7 and a cytokine gene could be incorporated in the future.
- 2) To confirm expression of these genes introduced directly into tumor cells *in vivo*.
- 3) To analyze the specificity of the immune response against this antigen *in vivo* by analyzing cellular and humoral immunity.

3.0 Selection of Patients

Patients will be carefully selected based on consideration of their past medical history and present status. The referring physician, the attending physician, and surgical oncology or the relevant clinical department, the patient, and family members will make a joint decision regarding the appropriate treatment with conventional therapy. If surgery, chemotherapy, or radiation are deemed unlikely to provide further benefit to the patient, the opportunity to pursue this experimental protocol will be offered to the patient. The following criteria will be used to select appropriate patients for study:

3.1 Criteria for Patient Eligibility

- 3.1.1 Patients must be HLA-B7 negative.
- 3.1.2 Patients with cutaneous tumor nodules will be preferred in patients undergoing intratumor injection.
- 3.1.3 The patient's disease will be unresponsive to standard modes of treatment.
- 3.1.4 Patient must be greater than 18 years of age.
- 3.1.5 The patient must not have antibodies to the human immunodeficiency virus.
- 3.1.6 The patient must be able to provide informed consent.
- 3.1.7 Estimated life expectancy >2 months.

3.2 Criteria for Patient Ineligibility

- 3.2.1 Patients with active autoimmune disease or infection.

- 3.2.2 Patients who have a positive antibody to HIV.
- 3.2.3 Patients with active hepatitis, chronic or acute.
- 3.2.4 Patients with diabetes mellitus who are not controlled by medical treatment.
- 3.2.5 Psychiatric illness which makes compliance to the clinical protocol unmanageable.
- 3.2.6 Patients with unstable angina or complications of cardiovascular disease which would contraindicate catheterization, if relevant.
- 3.2.7 Patients on high dose glucocorticosteroids.
- 3.2.8 Patients who undergo an alternative mode of anti-cancer treatment will not be accepted within 4 weeks of the last treatment.

4.0 Clinical Evaluation Prior to Treatment

As described above, patients will be selected based on their past medical history and clinical course. Patients must have histologically confirmed metastatic melanoma which is unresponsive to standard curative or palliative measures. Patients with expected survival times of 1 year or less will be chosen. The patients must meet the standard inclusion and exclusion criteria described above. In addition, the chosen patients must show no signs of active systemic infection or major cardiovascular or respiratory disease. The following laboratory values will be used as guidelines as enrollment:

1. The bilirubin should be less than 2 mg/dl. The platelet count should be greater than $100,000/\text{mm}^3$. The white blood cell count should exceed $3,000/\text{mm}^3$. The creatinine should be less than 2.0 mg/dl.
2. HLA typing must indicate that the patient is HLA-B7 negative.
3. HIV antibody titers will be measured.

5.0 Stratification and Randomization

Not relevant.

6.0 Nature of Procedures or Therapeutic Agents

6.1 Study Design

Patients will be admitted to the Clinical Research Center at The University of Michigan Medical Center after the relevant eligibility criteria have been met. The pre-treatment evaluation will be performed as described in Section 9. Patients undergoing the direct gene transfer protocol will have serum samples removed for analysis as described in Section 9. The tumor nodule to be injected will be identified and its borders measured prior to injection. A needle biopsy will be performed to confirm the diagnosis and to analyze as a pretreatment sample. Tissue will be stored as frozen sections for further immunohistochemical analysis and PCR. In addition, this nodule and other control (untreated) nodules will be imaged by CT immediately prior to the procedure, and its size quantitated. The skin overlying the tumor nodule will be sterilized and anesthetized using .01% lidocaine. For gene transfer, a 22-gauge needle will be used to inject the DNA liposome complex which will be prepared as follows: 10 minutes prior to delivery, 0.1 ml of plasmid DNA (.05-50 mg/ml) in lactated Ringer's solution is added to 0.1 ml of DMRIE/DOPE liposome solution (.15-15 μ M). Each component will be stored separately in sterile vials and certified as acceptable by the FDA. The solution is left at room temperature for 5-10 minutes and 0.8 ml of sterile lactated-Ringer's is added to the liposome DNA solution. The optimal composition of the DNA/liposome complex has been established for each batch by titration of DNA concentration and liposome concentration

independently on human melanoma and renal cell carcinoma *in vitro*, and confirmed by direct injection into melanoma or other tumors *in vivo* prior to use. Each component, the liposome preparation and the DNA, will be tested for contaminants and toxicity and used according to previously established guidelines from the FDA. The liposome solution and DNA will be aliquoted in individual sterile vials mixed under sterile conditions as described above.

For direct injections of the HLA-B7 plasmids, escalating doses will be studied in this phase I study. Four groups (3 patients each) will be studied sequentially with at least 1 month of observation prior to evaluation of the next group. Patients in each group will receive intratumor injections. Group I will receive 3 injections of 0.2 ml within the same nodule (3 μ g of DNA + 4.5 nM DMRIE/DOPE). Group II will receive the same treatment with a 10-fold higher concentration of DNA liposome complex. Group III will receive 100-fold higher dose, and Group IV will receive 1000 x higher amount. We are currently evaluating whether pre-treatment with low dose cytoxan can improve the anti-tumor response by eliminating suppressive T cells. If this approach is helpful, it will be included as part of the protocol.

For catheter-based gene delivery, the same dose escalation will be used, except a single 0.6 ml injection into the end artery which perfused an isolated nodule will be used with an occlusion balloon catheter. In murine and porcine models, the highest treatment exceeded these proposed doses by 100-fold and

are well-tolerated. Doses will be repeated within each subject for whom the toxicity treatment is with $\leq$ grade II. Dose escalation will begin if three patients show toxicities $<$ grade III from the treatment. If one patient displays toxicity $>$ grade II, the treatment will be repeated on three additional patients. If two patients develop toxicity $>$ grade II, the dosage will be reduced. The maximal tolerated dose will be defined as the dose at which two or more patients out of six develop grade III or IV toxicity. The treatment dose will be established at one level below the maximum tolerated dose. Once the treatment dose is defined, an additional four to six patients will be entered at that dose to ascertain the safety of this dose for wider application.

Prior to the injection with the needle in place, gentle aspiration will be applied to the syringe to ensure that no material is injected intravenously. Immediately after the injection procedure, a blood sample will be obtained to check serum enzymes, chemistries, and blood counts, and to analyze for the presence of plasmid DNA in the peripheral blood by PCR. The patients will be observed in the Clinical Research Center for an additional 48 hours, and another blood collection performed as described in Section 9. If there are no complications, the patient will be discharged after 48 hours. Should any abnormalities appear, the patient would be kept for further observation.

6.2 Confirmation of Gene Transfer and Expression

Needle biopsy of the injected nodule will be performed after administration of local anesthesia prior to injection and subsequently to treatment (preferably at day 3). A portion of this tissue will be processed to obtain DNA for PCR analysis. The remaining tissue will be processed for pathologic analysis and immunohistochemical and/or immunofluorescent staining. If sufficient material can be obtained, RNA PCR analysis will also be performed. For internal organs, CT or ultrasound guided thin needle biopsies will be obtained when possible.

6.3 Analysis of Immune Response

Evidence of gene transfer can also be obtained indirectly by examination of the specific immune response to HLA-B7. The analysis will be performed as follows: two weeks prior to the initial treatment, a blood sample will be obtained to derive lymphocytes which will be immortalized using the Epstein-Barr virus. An aliquot of these cells will be further infected with an amphotropic HLA-B7 retroviral vector, and expression will be confirmed on the cell surface. These cells will subsequently be used in the laboratory as target cells for the cytolytic T cell assay. At no time will these cells be brought into the same building where the patient is being treated. These cells and laboratory experiments will be performed in MSRB II, Room 3560, whereas the patient's clinical treatment will be taking place in the Clinical Research Center of The University of Michigan Medical Center Hospital.

6.4 Repeated Treatment

If no adverse side effects of the treatment are observed, repeated injections will be considered at two-week intervals. Doses identical to the initial treatment regimen will be repeated with similar protocols and observation as described in Section 6.1.

6.5 Postmortem Analysis

The life expectancy of patients who enter this protocol will be limited, in general, less than six months. Important information can be obtained by analysis of tissue postmortem, including presence of DNA in germ line tissue, distribution of DNA and immune function, and potential toxicity. To maximize the information derived from these studies, every effort will be made to perform postmortem analyses. Consent for an autopsy will not be required for entry, but patients and their families will be informed of this aspect of the study and its potential contribution to medical knowledge.

7.0 Schema/Duration

Tumor Injection

Group	No. of Injections Per Treatment	Volume of Injection (ml)	Times of Repeated Treatment	Total Number of Treatments	Dose DNA	Liposome
I	3	0.2 x 3	2 wk.	3	.003 mg	.0045 μ M
II	3	0.2 x 3	2 wk.	3	.03 mg	.045 μ M
III	3	0.2 x 3	2 wk.	3	.3 mg	.45 μ M
IV	3	0.2 x 3	2 wk.	3	3 mg	4.5 μ M

Catheter Delivery

Group	No. of Injections Per Treatment	Volume of Injection (ml)	Times of Repeated Treatment	Total Number of Treatments	Dose DNA	Liposome
I	1	0.6	2 wk.	3	.003 mg	.0045 μ M
II	1	0.6	2 wk.	3	.03 mg	.045 μ M
III	1	0.6	2 wk.	3	.3 mg	.45 μ M
IV	1	0.6	2 wk.	3	3 mg	4.5 μ M

8.0 Dose of Therapeutic Modifications

Patients can receive medications to control any side effects of treatment. Such agents would include acetaminophen (650 mg. q. 4 hrs.), indomethacin (50-75 mg. q. 6 hrs.), or antihistamines as required. Glucocorticoids will not be used in these patients; or if such medication is required, the patient will be removed from the protocol.

9.0 Study Parameters (revised)

Parameters

	<u>Pre-Study</u>	<u>During Treatment</u>					
		<u>Day</u>				<u>Week</u>	
		<u>1</u>	<u>3</u>	<u>14</u>	<u>28</u>	<u>6</u>	<u>8</u>
Physical exam	X	*		X	X	X	X
History	X						
Performance Status	X						X
Tumor Staging and/or Nodule Imaging	X			X	X	X	X
Chemistry Survey ¹	X	X	X	X	X	X	X
Vital Signs	X	X	X	X	X	X	X
Weight	X	X	X	X	X	X	X
CBC, Diff, Platelet	X	X	X	X	X	X	X
PT, PTT	X	X		X	X		X
EKG	X		X				
CPK + isoenzymes	X	X		X	X	X	X
CXR	X				X		X
U/A and Culture	X						
Hb _s Ag, HIV	X						
Brain CT or MRI	X						
Assess for adverse events status	X	X	X	X	X	X	X
PCR on PBL and serum to detect HLA-B7 gene	X	**X	X	X	X	X	X
Tumor biopsy	X			X	X	(X)	(X)
Assay for HLA-B7 cytotoxic T cells	X			X	X		X
Assay for HLA-B7 antibody	X			X	X		X
Pregnancy test (if relevant)	X						
Anti-DNA antibodies ds DNA ss DNA	X				X		X
CT scan of brain (if relevant)	X				X		X
CT of chest & abdomen	X					X	(X)
Quantitate size of lesion	X			X	X		X
ANA, ESR, CH50, CRP	X				X		X

* An additional sample will be taken several minutes after injection to examine for the presence of plasmid DNA.

** To be performed on nodules 1 week prior to treatment. Nodules will be between 0.25 and 4 cm in size.

¹ Routine laboratory tests include uric acid, calcium, phosphate, SGOT, SGPT, alkaline phosphatase, LDH, bilirubin, BUN, creatinine, total protein, glucose, amylase, lipase

9.1 Pre-treatment (see Table)

The following studies will be performed as summarized in Table 1.

- a. Complete physical examination noting in detail the exact size and location of any lesions that exist. The tumor lesion to be treated will be imaged and its dimensions quantitated prior to treatment.
- b. Complete chemistry survey including electrolytes, liver function tests, calcium, magnesium, creatinine, BUN, CPK, pancreatic enzymes.
- c. CBC, differential count, PT, PTT, platelet count.
- d. Urine analysis and culture.
- e. Hepatitis screen.
- f. HIV titer.
- g. Pregnancy test for women.
- h. Chest x-ray.
- i. Electrocardiogram.
- j. Baseline x-rays and nuclear medicine scans to evaluate the status of disease.
- k. CT scan or MRI scan of brain.
- l. 45 ml of clotted blood for serum storage and 45 ml of anti-coagulated blood for mononuclear cell cryopreservation.

- m. Biopsy of tumor, if possible with minimal morbidity.

9.2 Treatment

Patients will be analyzed with routine blood and chemistry analysis. A chest x-ray will be performed each month for six months and every six months thereafter or more often if needed. During the gene transfer protocol, patients will be monitored closely in the Clinical Research Center. Vital signs will be measured every 15 minutes prior to, during, and after the injection for at least two hours or until the patient is stable. If the systolic blood pressure drops below 80 mm/Hg, the injection will be terminated immediately.

9.3 Post-treatment

Cutaneous lesions will be evaluated by physical examination, biopsy if feasible, and appropriate imaging scans prior to injection, monthly for 6 months and six-month intervals or as needed to evaluate response to treatment. Serum will be analyzed by PCR for the presence of plasmid, and antibody to HLA-B7 will be evaluated. PBL's will be isolated and analyzed for their ability to lyse HLA-B7 modified autologous EBV-transformed lines. If ANA becomes positive or other signs of autoimmunity appear, evidence of more specific autoantibodies, e.g., anti-Rho, Smith, etc., will be tested and rheumatology consultation will be sought.

9.4 Potential Side Effects and Reporting of Adverse Reactions

Adverse Drug Reactions will be reported to the Drug Information Service, University of Michigan Medical Center, phone 313-936-8200 or 313-936-8251 (available 24 hours), and include all life-threatening events (Grade 4) which may be due to drug administration, all fatal events, or the first occurrence of any previously unknown clinical event (regardless of Grade). A written report is to follow within 10 working days to:

Investigational Drug Branch

P.O. Box 30012

Bethesda, Maryland 20824

Phone: 301-496-7957

Data will be submitted to the IRB yearly or upon request. All adverse reactions will be reported to the IRB, even if there is only a suspicion of a drug effect. All side effects will be graded using the standard toxicity sheet described in Section 14, Common Toxicity Criteria.

9.4.1 Potential Risks of Gene Transfer In Vivo

Insertional mutagenesis. The possibility of causing malignancy in cells secondary to the random insertion of the DNA in the genome exists, though this risk is considered low. There is a remote chance that the vector could replicate in the host. Neither finding has been seen in animal studies (~200 mice, rabbits, and pigs); nonetheless, the proposed protocol provides for extensive testing of tumor tissue and blood after injection

into the patient. PCR will be performed to monitor for this unlikely event.

The use of aminoglycoside antibiotics. The neomycin resistance gene, which encodes neomycin phosphotransferase (NPT), phosphorylates the 3' hydroxyl group of the aminohexose I of neomycin and its analogues, inactivating the antibiotic. While amikacin may be inactivated by this enzyme, gentamicin and tobramycin do not contain an hydroxyl at the 3' position and are not inactivated. Therefore, introduction of the NeoR gene would not exclude the use of aminoglycosides or any other conventional antibiotic that may be needed in the clinical management of these patients.

Although risks to the patient exist in this study, they would appear to be minimal, and the escalating dose nature of this study should minimize the risks to any individual patient. Because these patients have limited life expectancy from their advanced cancer, these risks are thought to be justified, considering the potential therapeutic benefit.

10.0 Off-Study Criteria

Patients will be removed from the study should any grade 3 or 4 toxicity develop which is not easily corrected. The toxicity sheet is included in Section 14.0, Common Toxicity Criteria). If two treatment-related deaths should occur, this protocol will be terminated.

11.0 Evaluation of Results

11.1 Criteria for Response

In this phase I study, the protocol will be considered successful if recombinant gene expression is achieved and an appropriate dosage established for effective gene transfer without toxicity. Confirmation of recombinant gene expression is described below. Additional evidence of successful gene transfer may also be obtained from immunologic analysis. A new CTL or antibody response to HLA-B7 will indicate successful gene expression and is described in Section 11.3. The ability to generate anti-tumor CTL will also be evaluated. Evidence for tumor regression will also be obtained. Complete tumor response is defined as the disappearance of all clinical evidence of disease for at least four weeks. Partial tumor response is defined as 50% or greater decrease of the sum of the products of perpendicular diameters of lesions lasting at least four weeks with no increase in existing lesions or appearance of new lesions. The response will also be considered positive for gene transfer if cytolytic T cell activity for HLA-B7 is obtained after this injection. Any patient having less than a partial response is considered to be non-responsive to treatment. Tumor dimensions will be assessed by imaging as detailed in Section 9.

11.2 Confirmation of Recombinant Gene Expression

Several independent techniques will be used to evaluate the presence and expression of the recombinant gene *in vivo*. We have used several monoclonal antibodies to HLA-B7 to detect the recombinant gene product *in vivo* by immunohistochemistry.

Fluorescence staining of freshly dispersed cells will also be evaluated. The presence of plasmid DNA will be confirmed by PCR of DNA from tumor tissue, peripheral blood lymphocytes, or in autopsy specimen tissue. If sufficient tissue is available, RNA will be isolated and examined for the presence of HLA-B7 mRNA by PCR or S1 nuclease analysis.

11.3 Analysis of Immune Response

Direct gene transfer and expression of the HLA-B7 gene may sensitize the patient to HLA-B7 and lead to the generation of an immune response to this antigen. Limiting dilution analysis (LDA) will be utilized to evaluate alterations in the frequency of helper and cytolytic T cells for HLA-B7 in the peripheral blood following direct gene transfer. Peripheral blood lymphocytes will be isolated and cryopreserved prior to, and at 4-week intervals, following the initial direct gene transfer. At the completion of treatment, samples of PBL from each time point will be simultaneously evaluated for responsiveness to HLA-B7 by culturing PBL, under LDA conditions, with autologous EBV-B cells transduced with the HLA-B7 gene. Antigen specific elaboration of IL-2 or generation of CTL to HLA-B7 positive target cells will be the indices evaluated in these studies. The presence of antibody will be evaluated by FACS analysis of a matched pair of HLA-B7⁺ or HLA-B7⁻ cell lines. In some instances, lymphocytes will be isolated directly from the tumor, expanded in tissue culture, and analyzed for cytolytic function. Tumor biopsies at 7-14 days after treatment will be analyzed by immunohistochemistry. If possible, we will attempt to expand draining lymph node T cells

or TIL cells to test their cytologic function. When possible, we will derive autologous cell lines to be used as targets in a ^{51}Cr release assay. Finally, every attempt will be made to excise tumor tissue prior to treatment for diagnosis, immunohistochemistry, and cryopreservation and to evaluate delayed type hypersensitivity reactions to the tumor before and after treatment.

11.4 Toxicity

Toxic side effects of treatment will be analyzed and classified by the common toxicity criteria (Section 13).

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C O N S E N T F O R M

(To be read by the Patient and explained
to the Patient by his or her Physician).

PROTOCOL: Immunotherapy for Cancer by Direct Gene Transfer into Tumors

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PROTOCOL NUMBER: IRB 93-129 DATE: _____

PATIENT NAME: _____ HOSPITAL NO.: _____

INTRODUCTION

We invite you to participate in a research study at the University of Michigan Medical Center. Several general principles apply to all who take part in any experimental studies:

- (1) Your participation in this study is voluntary
- (2) You may not personally benefit from this study,
but knowledge may be gained from it that will benefit others
- (3) You may withdraw from the study at any time for any reason
without jeopardizing your further care.

The nature of the study, the risks, inconveniences, discomforts, and other information are discussed in the following sections. Please do not hesitate to discuss any questions you have about this study with the physicians who explain it to you.

DESCRIPTION OF TREATMENT OR PROCEDURE TO BE UNDERTAKEN

As you are aware, you have cancer which cannot be cured by medicine, surgery, or radiation. In this study, treatments will be offered that may help to fight this disease in future patients. Because the treatment is experimental, you may not derive any direct benefit from it. The purpose of this study is to determine a safe and effective dose of a new treatment which will attempt to induce tumor regression. Because this is a new, experimental treatment, we will also be observing you to determine the side effects of the therapy. We will also monitor the effects of this treatment on the growth of your tumor.

By using techniques in the laboratory, it is now possible to prepare large amounts of human DNA or genetic material in bacteria. This DNA will

be mixed with fat bodies called liposomes, and we plan to transport the mixture into your tumor by needle injection. Once introduced into the tumor, the DNA produces proteins which stimulate tissue rejection. One protein -- known as HLA-B7 -- causes the cells which will contain it to be recognized as "foreign enemy" by your immune system and, in some cases, a second protein, called interleukin-2, will be made. This protein also causes activation of your immune system. The purpose of our study is to determine whether this treatment will induce the cells of your immune system, known as lymphocytes, to attack and kill your tumor. This type of therapy which stimulates your lymphocytes is called immunotherapy.

Alternative Therapies

There are no known cures for patients with your disease. Other alternative treatments available to you can control local symptoms. These include the delivery of x-ray treatment to sites of local disease to control pain, medication to control pain, and medical, surgical, or radiation treatment of any reversible complications. Experimental drugs are being evaluated at other centers to which you can be referred. Other experimental treatments are under investigation which attempt to stimulate your immune system to reject your tumor, and you can be referred to physicians who are conducting such trials. In contrast to this treatment, other protocols usually require removal of tumor cells or blood cells, which are taken to the laboratory for genetic manipulation, and subsequently returned to you by injection. In some cases, proteins are injected which can stimulate the immune system. You also have the option to receive no treatment at this time.

Procedures

Before receiving this treatment, you will receive many tests to see if you qualify for this study. These tests will be made either as an inpatient or an outpatient. These tests include: 1) blood tests 2) x-rays of the brain, chest, and abdomen and 3) a blood test for the antibodies to Human Immunodeficiency Virus (HIV), which causes AIDS. If you have antibodies to HIV, you may not participate in this study.

If you qualify for this study, we will inject a solution containing the DNA/liposome complex directly into a tumor nodule. The injections will be made under sterile conditions after providing a local anesthetic (xylocaine), and multiple areas within a single nodule will be injected up to 5 times. The time of treatment is usually ~30 minutes. The treatment will be repeated every 2 weeks for a total of three. Blood samples (between 1-10 tubes) will be obtained daily at first, then weekly for the 1st month, and biweekly for the next month. A CT scan will be performed before initiation of treatment and at the end of the 2-month study period. Your blood lymphocytes will be tested for their ability to respond to the HLA-B7 antigen. We will also examine your blood for evidence of toxicity from this treatment.

At different times in the protocol, tumor biopsies will be performed. This procedure involves the injection of a local anesthetic (xylocaine) under sterile conditions, followed by insertion of a needle into the tumor nodule and withdrawal of a sample of the tumor. This procedure will be performed prior to treatment and at intervals of 2 weeks up to 4 times.

RISKS AND SIDE EFFECTS

There are potential side effects and risks to this procedure. First, you may experience mild discomfort from needle injections or tumor biopsies. You may have mild discomfort and bleeding from the tumor biopsy. You will be given a local anesthetic to minimize the discomfort. Second, even though the DNA inserted into your tumor is considered harmless to you, events could occur within normal cells that allow them to become cancerous. Laboratory studies suggest that this possibility is unlikely. However, this is a new procedure and we do not know whether cells could become abnormal after long periods of time. In animal studies, we have not observed the development of cancer cells in any animals tested. Third, the inserted DNA will contain a gene that inactivates certain antibiotics in bacteria. This protein is not likely to be made in humans, and many other antibiotics that are not inactivated will be available and effective in treating any potential bacterial infections.

We emphasize that this procedure, called direct gene transfer, has never been used before in human patients. Because this procedure is new, it is possible that despite our extensive efforts, other unforeseen problems may arise, including the very remote possibility that death may occur.

You will undergo biopsy of tumor and other tissue, if available, on several occasions before and after injection. Blood and tissue specimens will be taken where possible to follow the duration and effects of HLA-B7 expression. If we are successful in this protocol, you will be immunized to the HLA-B7 protein. In the event that you should require an organ transplant, you would not be able to receive an organ from an individual who makes this protein, on average, ~15% of donors.

Follow-Up

After you receive the treatment, you will be discharged from the hospital if you have no other significant medical problems. You will be required to return to the University of Michigan Medical Center for follow-up studies described above. Tests used to decide if your tumor has responded to the therapy will be similar to those you had before beginning the therapy. If your disease recurs after treatment in this protocol, you will be eligible for other protocols and will receive treatment as indicated by your disease or referred elsewhere for such treatment. Because this form of therapy is new, unanticipated side effects that may cause your condition to deteriorate could be encountered. You will be closely monitored for such side effects.

Treatment will continue as long as there is sufficient possibility of response to warrant the risks and side effects encountered. Your physicians feel that the risks of your disease are much greater than the risks of the treatment as outlined above. Furthermore, your physicians have considered your individual situation and have concluded that, at this time, no other therapeutic approaches such as surgery, radiation therapy, or other chemotherapeutic treatments are clinically indicated as being

more effective. At some later time, should these alternatives be clinically indicated, they will be discussed with you because this study does not preclude their use.

Other Pertinent Information

1. Confidentiality. When results of a study such as this are reported in medical journals or at meetings, the identification of those taking part is withheld. Medical records are maintained according to current legal requirements, and are made available for review, as required by the Food and Drug Administration or other authorized users, only under the guidelines established by the Federal Privacy Act. A qualified representative of the National Institutes of Health may inspect patient and study records. This procedure may attract attention from the media. We will make every effort to protect your confidentiality. Because of media interest, however, there is a significant chance that information concerning you and your treatment will appear publicly without your consent.
2. Policy regarding research-related injuries. In the unlikely event of physical injury resulting from research procedures, the University will provide first-aid medical treatment. Treatment of injuries or side effects directly related to this experimental treatment will be provided at no cost to you. Additional medical treatment will be provided in accordance with the determination by the University of its responsibility to provide such treatment. However, the University does not provide compensation to a person who is injured while participating as a subject in research. If you have questions regarding additional medical treatments, you may call the Patient Staff Relations Office (313/763-5456).
3. You will not be paid to take part in this study.
4. Outpatient and inpatient studies will be ordered to determine your eligibility for study and whether you have had a response to the treatment. Clinic visits, laboratory tests, x-rays, and scans will not be free. Items which are not covered by insurance which relate to this research protocol will be covered by research grants. The cost of tests and treatments unrelated to this study will be handled as usual and will depend on whether or not you have insurance and what costs your insurance covers. Unfortunately, insurance coverage cannot be guaranteed for all tests and treatments; however, you may discuss this issue with the hospital financial office or your insurance company before you agree to participate.
5. This consent form does not include consent relating to the risks of any surgical procedures. Any surgical procedures performed will require a separate consent form.

Please understand that you are free to withdraw your consent to participate in this study at any time during treatment or follow-up and seek care from any physician with no loss of benefits or disruption in your care.

The team of physicians and nurses providing care in this study are very experienced. You must realize, however, that unforeseeable or unexplained risks are always possible when investigational therapies are undertaken.

If you have questions pertinent to this research, you should contact Gary J. Nabel, M.D., Ph.D., at 313/747-4798.

If you feel that you have a research-related injury, contact Gary J. Nabel, M.D., Ph.D., at 313/747-4798.

The following numbers are for your use if medical problems develop during treatment:

Office: 313/747-4798
Clinical Research Center: 313/936-8090
Voice Mailbox: 313/764-9121 (after office hours)
Doctor or Nurse: 313/936-6266; Paul Watkins, M.D.
(This is a hospital beeper; ask the paging operator to page #9128. Use the following numbers in case of an **Emergency Only**).
(Outside of regular office hours if the paging service won't do.)
Home: 313/995-5848; Dr. Gary J. Nabel

Questions on my rights as a patient may be directed to Ann Munro in the Patient/Staff Relations Office at 313/763-5456.

I have fully explained to the patient, _____, the nature of the treatment program described above and such risks as are involved in its performance.

Physician's Signature

I have been fully informed as to the procedures to be followed including those which are investigational, and have been given a description of the attendant discomforts, risk, and benefits to be expected, and the appropriate alternative procedures. I realize that, since my participation is voluntary, I can refuse this treatment without in any way prejudicing my future medical care. In signing this consent form, I agree to this method of treatment, and I understand that I will receive the best supportive care even if not receiving this protocol treatment. I also understand that my doctors can stop my treatment on this protocol if they feel the risks in my case have increased, over time, to exceed the potential benefits to me. I understand, also, that if I have any questions at any time, they will be answered. I have received a copy of this consent form.

I am not and will not become pregnant during this study.

I understand that the University will provide first-aid medical treatment in the unlikely event of physical injury resulting from research procedures. Treatment of injuries or side effects directly related to the experimental treatment will be provided at no cost to me. Additional

medical treatment will be provided in accordance with the University's determination of its responsibility to do so. The University does not, however, provide compensation to a person who is injured while participating as a subject in research.

I have not engaged in any other research projects within the past six (6) months [].

Within the past six (6) months, I have been involved in a study by Dr. _____.

I have [] have not [] been under the care of a physician within the past twelve (12) months.

Signature of Patient

Date

Witness

University of Michigan Medical School
Department of Internal Medicine
and the Clinical Research Center

C O N S E N T F O R M

(To be read by the Patient and explained
to the Patient by his or her Physician).

PROTOCOL: Immunotherapy for Cancer by Direct Gene Transfer into Tumors

PRINCIPAL INVESTIGATOR: Gary J. Nabel, M.D., Ph.D.

CO-PRINCIPAL INVESTIGATORS: Alfred E. Chang, M.D.
Elizabeth G. Nabel, M.D.
Gregory E. Plautz, M.D.
William Ensminger, M.D.

PROTOCOL NUMBER: IRB 93-129 DATE: _____

PATIENT NAME: _____ HOSPITAL NO.: _____

INTRODUCTION

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but knowledge may be gained from it that will benefit others
- (3) You may withdraw from the study at any time for any reason
without jeopardizing your further care.

The nature of the study, the risks, inconveniences, discomforts, and other information are discussed in the following sections. Please do not hesitate to discuss any questions you have about this study with the physicians who explain it to you.

DESCRIPTION OF TREATMENT OR PROCEDURE TO BE UNDERTAKEN

As you are aware, you have cancer which cannot be cured by medicine, surgery, or radiation. In this study, treatments will be offered that may help to fight this disease in future patients. Because the treatment is experimental, you may not derive any direct benefit from it. The purpose of this study is to determine a safe and effective dose of a new treatment which will attempt to induce tumor regression. Because this is a new, experimental treatment, we will also be observing you to determine the side effects of the therapy. We will also monitor the effects of this treatment on the growth of your tumor.

By using techniques in the laboratory, it is now possible to prepare large amounts of human DNA or genetic material in bacteria. This DNA will

be mixed with fat bodies called liposomes, and we plan to transport the mixture into your tumor by needle injection. Once introduced into the tumor, the DNA produces proteins which stimulate tissue rejection. One protein -- known as HLA-B7 -- causes the cells which will contain it to be recognized as "foreign enemy" by your immune system and, in some cases, a second protein, called interleukin-2, will be made. This protein also causes activation of your immune system. The purpose of our study is to determine whether this treatment will induce the cells of your immune system, known as lymphocytes, to attack and kill your tumor. This type of therapy which stimulates your lymphocytes is called immunotherapy.

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There are no known cures for patients with your disease. Other alternative treatments available to you can control local symptoms. These include the delivery of x-ray treatment to sites of local disease to control pain, medication to control pain, and medical, surgical, or radiation treatment of any reversible complications. Experimental drugs are being evaluated at other centers to which you can be referred. Other experimental treatments are under investigation which attempt to stimulate your immune system to reject your tumor, and you can be referred to physicians who are conducting such trials. In contrast to this treatment, other protocols usually require removal of tumor cells or blood cells, which are taken to the laboratory for genetic manipulation, and subsequently returned to you by injection. In some cases, proteins are injected which can stimulate the immune system. You also have the option to receive no treatment at this time.

Procedures

We would like to deliver this treatment through a tube or catheter in your blood vessels. In this procedure, a small tube (catheter) is introduced into one or several of your blood vessels. Through this tube, a solution containing iodine (contrast medium) will be injected, which will enable us to see the blood vessels of the tumor on x-rays. After the vessel which supplies a tumor nodule in your lungs is identified, we will inject the genetic material complexed to fat particles that will allow HLA-B7 protein to be made in an attempt to stimulate your body to attack this nodule. The tube is introduced into the blood vessels, either in your arm or your groin, by means of minor surgery under local anesthesia. The entire procedure will take 1-3 hours to perform, of which the gene transfer will take ~15 minutes. It will be repeated at 2-week intervals for a total of three times. Although the possibility of clotting the vessel used is small, it does happen occasionally. In addition, it is possible that an artery or arteries feeding an organ could also be clotted. In either of these circumstances, it may be necessary to perform surgery to remove the clot or to treat you with certain medications which may dissolve the clot. We are sure you realize that although the risk is very small, clotting the blood supply to an organ can result in the loss of that organ. The overall serious complication rate is about 1 in 500 patients. Very rarely, a serious complication can result in death.

During the procedure, it is possible that the contrast medium might result in an adverse reaction, causing hives, shortness of breath, extremely low blood pressure, and very rarely, temporary or permanent _

paralysis. Also, if the procedure involves the heart or lungs, an abnormal heart rhythm may occur, requiring treatment.

The usual complications, which we would consider minor but which nevertheless can be distressing to the patient, are accumulation of blood in the tissues where the catheter has been introduced (hematoma), or a small bulge of the artery at the site where it was entered by the catheter. If you desire, these will be discussed with you.

Unfortunately, this information may have alarmed you, but we believe it to be in your best interest to understand what is involved. Any questions you have about the above material can be directed to Dr. Nabel, or the physician or the radiologist performing the angiogram. Before proceeding, we request your authorization as indicated below.

Before receiving this treatment, you will receive many tests to see if you qualify for this study. These tests will be made either as an inpatient or an outpatient. These tests include: 1) blood tests 2) x-rays of the brain, chest, and abdomen and 3) a blood test for the antibodies to Human Immunodeficiency Virus (HIV), which causes AIDS. If you have antibodies to HIV, you may not participate in this study.

If you qualify for this study, we will inject a solution containing the DNA/liposome complex directly into a tumor nodule. The injections will be made under sterile conditions after providing a local anesthetic (xylocaine), and multiple areas within a single nodule will be injected up to 10 times. Blood samples (between 1-10 tubes) will be obtained daily at first, then weekly for the 1st month, and biweekly for the next month. Your blood lymphocytes will be tested for their ability to respond to the HLA-B7 antigen. We will also examine your blood for evidence of toxicity from this treatment.

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will be available and effective in treating any potential bacterial infections.

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You will undergo biopsy of tumor and other tissue, if available, on several occasions before and after injection. Blood and tissue specimens will be taken where possible to follow the duration and effects of HLA-B7 expression. If we are successful in this protocol, you will be immunized to the HLA-B7 protein. In the event that you should require an organ transplant, you would not be able to receive an organ from an individual who makes this protein, on average, ~15% of donors. During the catheter procedure, there are several risks, including the potential for clot formation or a reaction to intravenous contrast. These risks are described more fully in the previous section entitled "Procedures."

Follow-Up

After you receive the treatment, you will be discharged from the hospital if you have no other significant medical problems. You will be required to return to the University of Michigan Medical Center for follow-up studies described above. Tests used to decide if your tumor has responded to the therapy will be similar to those you had before beginning the therapy. If your disease recurs after treatment in this protocol, you will be eligible for other protocols and will receive treatment as indicated by your disease or referred elsewhere for such treatment. Because this form of therapy is new, unanticipated side effects that may cause your condition to deteriorate could be encountered. You will be closely monitored for such side effects.

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patient and study records. This procedure may attract attention from the media. We will make every effort to protect your confidentiality. Because of media interest, however, there is a significant chance that information concerning you and your treatment will appear publicly without your consent.

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Please understand that you are free to withdraw your consent to participate in this study at any time during treatment or follow-up and seek care from any physician with no loss of benefits or disruption in your care.

The team of physicians and nurses providing care in this study are very experienced. You must realize, however, that unforeseeable or unexplained risks are always possible when investigational therapies are undertaken.

If you have questions pertinent to this research, you should contact Gary J. Nabel, M.D., Ph.D., at 313/747-4798.

If you feel that you have a research-related injury, contact Gary J. Nabel, M.D., Ph.D., at 313/747-4798.

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Doctor or Nurse: 313/936-6266; Paul Watkins, M.D.
(This is a hospital beeper; ask the paging operator to page #9128. Use the following numbers in case of an **Emergency Only**).
(Outside of regular office hours if the paging service won't do.)
Home: 313/995-5848; Dr. Gary J. Nabel

Questions on my rights as a patient may be directed to Ann Munro in the Patient/Staff Relations Office at 313/763-5456.

I have fully explained to the patient, _____, the nature of the treatment program described above and such risks as are involved in its performance.

Physician's Signature

I have been fully informed as to the procedures to be followed including those which are investigational, and have been given a description of the attendant discomforts, risk, and benefits to be expected, and the appropriate alternative procedures. I realize that, since my participation is voluntary, I can refuse this treatment without in any way prejudicing my future medical care. In signing this consent form, I agree to this method of treatment, and I understand that I will receive the best supportive care even if not receiving this protocol treatment. I also understand that my doctors can stop my treatment on this protocol if they feel the risks in my case have increased, over time, to exceed the potential benefits to me. I understand, also, that if I have any questions at any time, they will be answered. I have received a copy of this consent form.

I am not and will not become pregnant during this study.

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I have not engaged in any other research projects within the past six (6) months [].

Within the past six (6) months, I have been involved in a study by Dr. _____.

I have [] have not [] been under the care of a physician within the past twelve (12) months.

Signature of Patient

Date

Witness

4/93

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

CVs

NAME	POSITION TITLE		
Gary J. Nabel, M.D., Ph.D.	Associate Professor Internal Med. & Biological Chemistry		
EDUCATION (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)			
INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
Harvard College, Boston, MA	B.A.	1975	Biochemistry
Harvard University, Boston, MA	Ph.D.	1980	Cell & Dev. Bio.
Harvard Medical School, Boston, MA	M.D.	1982	Medicine
Brigham & Women's Hospital, Boston, MA	Resident	1985	Internal Medicine
Whitehead Institute, MIT, Cambridge, MA	Fellow	1987	Molecular Biology

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

PROFESSIONAL EXPERIENCE

1976-1980 Member, Board of Freshman Proctors and Advisors, Harvard College
 1976-1981 Officer of Harvard University, Boston, MA
 1980-1981 Instructor, Biology 99, Harvard University, Boston, MA
 1980-1983 Resident Tutor in Biology, Dunster House, Harvard University, Boston
 1980-1984 Fellow, Laboratory of Immunopathology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA
 1983-1985 Clinical Fellow in Medicine, Harvard University, Boston, MA
 1983-1985 Intern and Resident, Internal Medicine, Brigham and Women's Hospital, Boston, MA
 1984-1987 Instructor, Harvard Medical School, Boston, MA
 1985-1987 Associate, Howard Hughes Medical Institute, Whitehead Institute, MIT, Laboratory of David Baltimore
 1985-1987 Associate Physician, Brigham and Women's Hospital, Boston, MA
 1987-1990 Assistant Professor, Internal Medicine and Biological Chemistry, University of Michigan Medical Center, Ann Arbor, MI
 1987-1991 Assistant Investigator, Howard Hughes Medical Institute, University of Michigan Medical Center, Ann Arbor, MI
 1990-present Associate Professor, Internal Medicine (with tenure) and Biological Chemistry, University of Michigan Medical Center, Ann Arbor, MI
 1991-present Associate Investigator, Howard Hughes Medical Institute, University of Michigan Medical Center, Ann Arbor, MI

HONORS AND AWARDS

1971-1975 Harvard National Scholar
 1975 Magna Cum Laude, Harvard College
 1975 Mallinkrodt Book Prize
 1976-1982 Harvard Graduate National Scholar
 1982 James Tolbert Shipley Prize for Research, Harvard Medical School
 1992 Off. Citation, CT State Gen. Assembly for Contributions to Hum. Gene Therapy
 1992 Midwest AFRC Young Investigator Award
 1992-present American Society for Clinical Investigation

PUBLICATIONS (24 of 91 total publications)

1. Nabel G, Fresno M, Chessman A, and Cantor H. Use of cloned populations of mouse lymphocytes to analyze cellular differentiation. Cell 23:19-28, 1981.
2. Nabel G, Galli SH, Dvorak AM, Dvorak HF, and Cantor H. Inducer T lymphocytes synthesize a factor that stimulates proliferation of cloned mast cells. Nature 291:332-334, 1981.
3. Galli SH, Dvorak AM, Ishizaka T, Nabel G, DerSimonian H, Cantor H, and Dvorak HF

- cloned cell with natural killer function resembles basophils by ultrastructure and expresses IgE receptors. *Nature* 298:288-290, 1982.
4. Fresno M, DerSimonian H, Nabel G, and Cantor H. Proteins synthesized by inducer T cells: evidence for a mitogenic peptide shared by inducer molecules that stimulate different cell types. *Cell* 30:707-713, 1982.
 5. Yokota T, Lee F, Rennick D, Hall C, Arai N, Mosmann T, Nabel G, Cantor H, and Arai K. Isolation and characterization of a mouse cDNA clone that expresses mast cell growth factor activity in monkey cells. *Proc. Natl. Acad. Sci.* 81:1070-1074, 1984.
 6. Rennick D, Lee F, Yokota T, Arai K, Cantor H, and Nabel GJ. A cloned MCGF cDNA encodes a multilineage hematopoietic growth factor: multiple activities of interleukin-3. *J. Immunol.* 135:910-914, 1985.
 7. Nabel GJ. Order and human biology. *Am. J. Med.* 78:545-548, 1985.
 8. Mathey-Prevot B, Nabel G, Palacios R, and Baltimore D. Abelson virus abrogation of IL-3 dependence in a lymphoid cell line. *Mol. Cell. Bio.* 6:4133-4135, 1986.
 9. Nabel G and Baltimore D. An inducible transcription factor activates human immunodeficiency virus expression in T cells. *Nature* 326:711-713, 1987.
 10. Nabel GJ, Gorka C, and Baltimore D. T-cell-specific expression of interleukin 2: evidence for a negative regulatory site. *Proc. Natl. Acad. Sci. USA.* 85:2934-2938, 1988.
 11. Nabel GJ, Rice S, Knipe D, and Baltimore D. Alternative mechanisms for activation of human immunodeficiency virus enhancer in T cells. *Science* 239:1299-1302, 1988.
 12. Leung K and Nabel GJ. HTLV-I transactivator induces interleukin-2 receptor expression through an NF- κ B-like factor. *Nature* 333:776-778, 1988.
 13. Osborn L, Kunkel S, and Nabel GJ. Tumor necrosis factor- α and interleukin-1 stimulate the human immunodeficiency virus enhancer by activation of the nuclear factor κ B. *Proc. Natl. Acad. Sci. USA* 86(7):2336-2340, 1989.
 14. Griffin GE, Leung K, Folks TM, Kunkel S, and Nabel GJ. Activation of HIV gene expression during monocyte differentiation by induction of NF- κ B. *Nature* 339:70-73, 1989.
 15. Nabel EG, Plautz G, Stanley JC and Nabel GJ. Recombinant gene expression in vivo within endothelial cells of the arterial wall. *Science* 244:1342-1344, 1989.
 16. Bielinska A, Shivdasani RA, Zhang L, and Nabel GJ. Regulation of gene expression with double-stranded phosphorothioate oligonucleotides. *Science* 250:997-1000, 1990.
 17. Markovitz DM, Hannibal M, Perez VL, Gauntt C, Folks TM, and Nabel GJ. Differential regulation of human immunodeficiency viruses: a novel HIV-2 regulatory element responds to stimulation of the T-cell antigen receptor. *Proc. Natl. Acad. Sci. USA* 87:9098-9102, 1990.
 18. Nabel EG, Plautz G, and Nabel GJ. Site-specific gene expression in vivo by direct retroviral infection or transfection of the arterial wall. *Science* 249:1285-1288, 1990.
 19. Schmid RM, Perkins ND, Duckett CS, Andrews PC, and Nabel GJ. Cloning of an NF- κ B subunit which stimulates HIV transcription in synergy with p65. *Nature* 352:733-736, 1991.
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HUMAN GENE TRANSFER PROTOCOL

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*Gene Therapy for Gaucher Disease: Ex Vivo Gene Transfer and Autologous
Transplantation of CD34(+) Cells.*

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Gaucher disease is the most common lysosomal storage disorder. It is due to an inborn error of glycosphingolipid metabolism resulting from mutations in the gene coding for glucocerebrosidase (GC) (E.C. 3.2.1.45). The GC gene has been mapped to chromosome 1q21. More than 20 different mutations have been described in the human GC gene. Two common mutations account for 80% of the mutant alleles. A correlation between the genotype and clinical phenotype permits separation of the rare neurodegenerative cases from the more common non-neuronopathic disease. The latter, Type 1 disease, occurs commonly among Ashkenazi Jews where it is the most prevalent inherited disorder. Type 1 disease accounts for 90% of the clinical cases of Gaucher disease. All patients with Gaucher disease have clinical complications frequently leading to discomfort and disability. Complications include hepatosplenomegaly, hypersplenism, anemia, thrombocytopenia and degenerative changes in the skeleton. Many patients have aggressive disease with clinical presentation early in life marked by profound organomegaly, bleeding diatheses, liver failure, esophageal varices, pulmonary compromise, pathologic fractures, bone deformity, and an early death.

There is a need for new forms of therapy to correct this genetic defect. Current treatments are either unavailable or prohibitively expensive. The objective of this proposal is to evaluate gene therapy for Type 1 Gaucher disease. We propose to perform autologous bone marrow transplantation with CD34⁺ cells transduced by the normal human GC gene. Preclinical data in the mouse model of bone marrow transplantation provides evidence of efficient transduction of bone marrow stem cells and long term expression of the human gene in their progeny. Expression of the transferred human GC gene in macrophages derived from the bone marrow of these animals endures for the life of the mice and is about 4 times the endogenous activity. In Gaucher disease, storage of the undegraded lipid occurs only in this cell type and the pathology of the disorder is mediated by macrophages. Preclinical studies with macrophages from Gaucher patients indicate that transduction of human Gaucher macrophages in culture is sufficient to correct their activity to within the normal range. These results suggest that gene transfer and expression of the GC enzyme in human macrophages is feasible. We have provided evidence that enzymatically competent macrophages are sufficient to treat the disease by either allogeneic transplantation of normal bone marrow or direct correction of macrophage enzymatic activity. We propose to genetically correct CD34⁺ cells capable of reconstituting the whole BM including MØ. Transfer of the GC gene to CD34⁺ cells obtained from blood of a Gaucher patient demonstrates a transduction efficiency of approximately 20% and expression of enzyme activity to 20-40 times the amount of activity in enzyme deficient cells.

We will transduce CD34⁺ cells obtained from the blood of Gaucher patients using a replication defective retroviral vector called R-GC. The vector carries the human GC cDNA. Five (5) patients will be studied over the first year of the study. Patients will be transplanted with autologous CD34⁺ cells that have been genetically corrected. The transplanted patient will be assessed for the carriage and expression of the transduced gene in peripheral blood leukocytes. GC activity will be used to quantify the extent of restoration of enzyme in these cells. The criteria of clinical responsiveness will be the ability to withdraw enzyme following the transplantation of patients previously treated with enzyme or clinical reversal of symptoms in the patient not receiving enzyme. Clinical parameters to be followed include organ size, hemoglobin/hematocrit, platelet count, plasma angio-tensin converting enzyme concentration, serum non-tartrate inhibitable acid phosphatase, bone marrow morphology and bone structure by x-rays and scans.

Gaucher disease is an inherited disease. Patients suffer from enlarged organs, bone deterioration with multiple fractures and in some patients, progressive neurological degeneration. Most patients suffer significant disability and, many, an early death. The symptoms of Gaucher disease are a consequence of the accumulation of a fatty substance within specialized house keeping cells called macrophages(MØ). Bone marrow transplantation (BMT) results in the replacement of sick MØ by normal ones. It has resulted in significant clinical improvement for some patients. Unfortunately the therapy is not available for the majority of patients because they lack a suitable bone marrow donor. The development of macrophage targeted enzyme replacement therapy has provided treatment options, but is not a completely satisfactory solution. Although patients improve with enzyme treatment, the therapy is not permanent and is very expensive. The cost for an adult usually exceeds \$250,000 per year per patient.

Gene therapy is a novel approach to the treatment of genetic diseases. It involves the addition of a normal gene to the cells of a patient with an inherited defect in the corresponding gene. Bone marrow cells are an important target cell for gene transfer in Gaucher disease because MØ are derived from the bone marrow. In the proposed approach to treatment, cells that can make the bone marrow would be collected from the patient, genetically corrected by inserting the normal gene, and then re-colonized in the patient's bone marrow. This is called gene transfer and autologous BMT. It avoids the immunologic problems of graft rejection and graft-versus-host disease, which occur with high frequency in bone marrow transplantations from a donor.

To be successful, gene therapy requires high efficiency gene transfer into cells, followed by the sustained activity of the transferred gene to do the job of making the enzyme. The most efficient way to transfer and express genes in mammalian cells is by the use of modified viruses acting as carriers of genes. These are called vectors. The most often used and most completely studied vectors are derived from retroviruses. Retroviral vectors use the natural ability of these life forms to infect cells and integrate their genetic material into the target cell's chromosomes. By this action, these vectors provide cells with potentially therapeutic genes. Retroviral vectors can be rendered unable to multiply themselves and still retain the ability to transfer genes into cells of many different types leading to stable, intact residence in the host cell DNA.

Correction of the genetic defect in bone marrow producing cells could result in the life-long production of enzymatically competent MØ. This should be therapeutic for patients with GD.

We have isolated the gene for glucocerebrosidase(GC) and demonstrated that transfer of the gene reversed the enzyme deficiency in the cells of patients with Gaucher disease. We have shown in bone marrow transplantation studies in mice that a retroviral vector can efficiently transfer the GC gene to primitive cells in bone marrow. Moreover, the human gene produced the GC enzyme in macrophages for the life of the mice. Furthermore, we have shown that transfer of the GC gene to macrophages obtained from the blood of Gaucher patients completely corrected the deficiency of the enzyme (GC) in this important cell. We also have transferred the normal human GC gene to cells from human blood enriched for bone marrow producing cells (CD34⁺) capable of reconstituting the bone marrow. The transduced cells expressed 2-4 times the amount of GC enzyme normally present in these cells. Recent results reveal that the CD34⁺ cells from a Gaucher patient are also corrected to 2-4 times the normal amount of enzyme. This is an important advance because the correction occurs in a cell which can re-establish the entire bone marrow including macrophages. The success of these studies permit consideration of clinical trials of gene therapy for patients with Gaucher disease.

CLINICAL PROTOCOL

Gene Therapy for Gaucher Disease: Ex Vivo Gene Transfer and Autologous Transplantation of CD34⁺ Cells

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III. BACKGROUND

A. GAUCHER DISEASE

The lysosome is an organelle containing the enzymatic machinery needed to degrade macromolecules to soluble products which may be either excreted or reutilized⁽¹⁾. Inherited mutations in the genes coding lysosomal enzymes result in deficiencies of these activities and consequent storage of their substrates within the organelle⁽²⁻⁶⁾. In patients with Gaucher disease, a lysosomal β -glucosidase is deficient which results in the lysosomal storage of a complex glycolipid called glucosylceramide (trivial names: glucosylceramide = glucocerebroside; acid- β -glucosidase = glucocerebrosidase)⁽⁷⁻⁸⁾.

On the basis of clinical signs and symptoms, Gaucher disease has been divided into three subtypes: type 1 (nonneuronopathic), type 2 (acute neuronopathic), and type 3 (subacute neuronopathic). All three types of Gaucher disease are caused by a deficiency of glucocerebrosidase resulting in the accumulation of glucosylceramide within the cells of the reticuloendothelial system⁽⁹⁾. The principal difference between the subtypes is the presence and progression of neurologic complications. A summary of subtypes of Gaucher disease and a review of the disorder is provided in the appendix.

B. GLUCOCEREBROSIDASE

Glucocerebrosidase (E3.2.1.45) (GC) is a monomeric, membrane - associated, hydrophobic glycoprotein with a molecular mass of 65000 daltons. Human GC protein consists of 497 amino acids. The enzyme is translated as a precursor protein with a 19 amino acid hydrophobic signal peptide which directs its co-translational insertion into the lumen of the endoplasmic reticulum-golgi-lysosome complex⁽¹⁰⁾. GC acts at the acidic pH of the lysosomal environment to hydrolyze beta-glucosidic linkages in complex lipids. The catalytic activity is increased by detergents, lipids and a naturally occurring activator protein known as sphingolipid activator protein-2 (SAP-2 or saposin C)^(11,12). Human GC cDNA was first cloned from a hepatoma cDNA library expressed in lambda gtl1 by screening with a polyclonal antibody developed to purified human placental GC⁽¹³⁾. Subsequent characterizations of a number of separately isolated GC CDNA clones have described the complete nucleotide sequence^(14,15). The glucocerebrosidase gene has been localized to human chromosome 1 q21 by in situ hybridization⁽¹⁶⁾. Analysis of genomic clones has shown the GC gene to consist of 11 exons and 10 introns spanning approximately 7KB. A pseudogene is present in tandem with the active gene⁽¹⁶⁻¹⁸⁾.

Deficiency of glucocerebrosidase is the cause of Gaucher disease^(7,8). More than twenty mutations in the gene are known, but only two are common⁽¹⁹⁾. These two account for approximately 80% of the mutant alleles⁽²⁰⁾. A third mutation has recently been reported and is believed to occur in 20% of type 1 Gaucher patients⁽²¹⁾. The several diseases grouped under the eponym of GD are inherited in an autosomal recessive pattern and each disease is caused by a deficiency in the lysosomal acid hydrolase responsible for the catabolism of glucosylceramide⁽⁹⁾. Although glucocerebrosidase is deficient in all cells, for unknown reasons, the accumulation of glucosylceramide occurs virtually only in macrophages. Storage within only one cell type is unique among the lysosomal storage diseases. This characteristic of the pathobiology of Gaucher disease has allowed the development of two treatment strategies based on the correction of enzyme deficiency in macrophages. The first approach to be of clinical benefit was allogeneic bone marrow transplantation which results in the repopulation of affected tissues with enzyme-competent macrophages^(22,23). Transplanted patients improve after engraftment of donor marrow and gradually resolve their organomegaly⁽²⁴⁾. The second approach to result in clinical improvement in Gaucher disease patients was macrophage-targeted enzyme replacement⁽²⁶⁾. This method took advantage of naturally occurring mannose receptors on macrophages and the exposition of accessible mannose residues in the oligosaccharides of glucocerebrosidase to effect an efficient, targeted delivery of the enzyme to macrophages^(25,26). Patients have responded to this

treatment and many of the related clinical problems have resolved^(27,28). Both of these approaches are novel and are important because they provide some means of therapy where none previously existed. However, both approaches have significant limitations. Allogeneic bone marrow transplantation carries morbidity and mortality risks that are unacceptable for many patients. Furthermore, matched donors do not exist for the majority of patients. Macrophage-targeted enzyme replacement is a life-long treatment and extremely expensive therapy. Current costs are between \$150,000 and \$450,000 per year per patient. Clearly, some alternative treatment is required for this disease.

Despite their limitations, the success of these strategies has demonstrated that enzymatic correction of only one cell type - the macrophage - results in effective therapy. This is important from the point of view of developing gene therapy for Gaucher disease because these approaches demonstrate the potential efficacy of gene therapy. The fact that marrow transplantation is effective allows one to consider hematopoietic stem cells (HSC) as an appropriate target cell for gene transfer. Successful transduction of a sufficient number of HSC could provide a life-long cure of the disease. Furthermore, because expression of the GC gene in the macrophage lineage alone may be sufficient for therapy, one could consider, as less permanent alternatives, gene transfer to committed macrophage precursors, peripheral blood monocytes, or to cultures of bone marrow capable of producing enzyme competent macrophage precursors.

C. GENE TRANSFER/GENE THERAPY

To be an effective permanent treatment, gene therapy requires the transfer and sustained expression of genes in cells important to the pathogenesis of the disease. Sufficient expression of a transduced glucocerebrosidase gene in the progeny of pluripotent bone marrow stem cells would correct the deficiency of the enzyme in all cell lineages including monocytes/macrophages. Experience from allogeneic marrow transplantation and macrophage-targeted enzyme replacement supports the idea that gene therapy would be curative provided adequate expression of the GC gene were accomplished in a sufficient number of macrophages. Much experience has been gained with systems to evaluate the efficiency of gene transfer and expression in hematopoietic cells using retroviral vectors^(29,30). Most of these studies have been conducted in mice where conditions to accomplish efficient gene transfer to hematopoietic stem cells have been defined⁽³¹⁻⁴¹⁾. Recent data suggest that a high percentage of stem cells can be transduced and survive to repopulate marrow⁽⁴⁰⁾. For the mouse, critical parameters for efficient retroviral gene transfer and repopulation of bone marrow stem cells include high titer virus producer cell lines (VPL), pretreatment of mice with 5-fluorouracil (5-FU) to initiate stem cell cycling⁽³⁸⁻⁴⁰⁾, pre-culture of bone marrow with growth factors including IL-3 and IL-6⁽⁴³⁾, and extended infection by coculture of the cells with the VPL for 2-6 days with growth factors⁽⁴⁰⁾. Several studies have shown that the GC gene can be transferred to mouse bone marrow cells, but only two studies have demonstrated sustained expression in animals⁽⁴¹⁻⁴⁷⁾.

One of the early problems encountered in the transfer and expression of genes in bone marrow has been the low transduction efficiency of cells in the bone marrow capable of hematopoietic reconstitution of the animal⁽⁴⁸⁻⁵⁸⁾. Improvements in gene transfer vectors and advances in the knowledge of the biology of the bone marrow, particularly in the use of cytokines to maintain cycling stem cells, has improved the ability to efficiently transfer genes to hematopoietic cells⁽⁵⁹⁻⁶⁴⁾. While much of this information has been derived from and may be applicable only in murine systems, the factors important to the transfer of genes to cells capable of hematopoietic reconstitution in larger animal species including man are being defined⁽⁶⁵⁻⁷¹⁾. Expression of transferred genes *in vivo* for long periods of time has been more problematic. The reasons for inconsistent expression of the transferred gene are not well understood but hypotheses include promoter suppression in gene transfer constructs with multiple promoters⁽⁷²⁾, down regulation of heterologous viral promoters⁽⁷³⁻⁷⁴⁾, and clonal loss of transduced long term repopulating cells⁽⁷⁵⁾. Despite these apparent obstacles, some

recent studies suggest that the problem of expression of transferred genes is being overcome in a variety of systems^(59,65,74-78).

Clinical studies of gene therapy are increasing rapidly. Evaluation of the safety and effectiveness of the approach are the subject of intense investigation⁽⁷⁹⁾.

IV. PRE-CLINICAL STUDIES

A. DEFINITION OF THE TARGET CELL AND EFFICACY OF THE GENE PRODUCT

As the first step toward this goal, we cloned the gene for glucocerebrosidase^(13,15). We demonstrated that the cDNA in a retroviral vector, pWE-GC, provided by Dr. Richard Mulligan, could correct the deficiency of GC in fibroblasts from a patient with Gaucher disease^(80,81). Earlier we had demonstrated that allogeneic bone marrow transplantation resulted in the reversal of the disease process⁽²³⁾. These results supported the hypothesis that gene therapy might be used to treat the disease by transfer of the GC gene to bone marrow cells. In addition, we demonstrated that targeting GC enzyme to macrophages resulted in a clinical response in a patient with Gaucher disease^(9,26,27). This result supports the hypothesis that the gene product is therapeutic. These experimental approaches also provided some encouragement that reconstitution of only a part of the normal enzyme capacity of a tissue would be sufficient to treat the disease. We concluded from these studies that gene therapy was possible as a treatment for Gaucher disease, provided that a fraction of tissue macrophages could be transduced with and would express the GC gene.

B. VECTOR CONSTRUCTIONS

The gene therapy approach to Gaucher disease required a vector that would result in efficient transduction and expression in hemopoietic cells. Dr. Donald Kohn joined the group to work on this project. He constructed a set of retroviral vectors derived from the Moloney murine leukemia virus (MMLV) placing the GC cDNA under the control of either its own promoter or one of several strong heterologous promoters. These N2 derived vectors were replication defective and were raised in the ecotropic virus producer cell lines to titers of 10^5 to 10^7 . Viral supernatants were used to infect 3T3 cells to test the vectors for efficiency of infection and expression of the GC gene. In 3T3 cell targets, transduction efficiency for all the vectors was essentially the same and approached 100%. The vector giving the most consistent results in terms of expression of the GC gene was a construct called N2-SV-GC. This vector resulted in about a two fold increase above control in human GC RNA transcripts in Northern blots or in GC protein on Western blots using a human specific monoclonal antibody (Mab-8E4), or in activity of glucocerebrosidase in extracts. This expression surpassed that obtained from vectors using either homologous GC gene promoter regions or the HSV-TK promoter⁽⁸²⁾. N2-SV-GC was used to study the transfer of the human gene to cells growing in long term bone marrow cultures (LTBMC)⁽⁴¹⁾. The goal of these experiments was threefold; first, to obtain a supply of transduced stem cells from LTBMC which could be used to study GC gene transfer in mice with eventual application to the GD mouse model. The second purpose was to study methods to support and expand cycling stem cells in culture as a source of marrow repopulating cells that might be adapted later for human applications. Third, the plan was to demonstrate that transduction of human Gaucher bone marrow by a normal GC gene would correct the enzyme deficiency in HSC marrow cells and their progeny.

The results of studies of N2-SV-GC in LTBMC demonstrated that N2-SV-GC was capable of infecting LTBMC. The transduction efficiency was high and expression of the GC gene could be detected in some spleen 'foci' in mice transplanted with bone marrow infected by N2-SV-GC. The amount of expression approached that seen in normal fibroblasts. These experiments demonstrated that the first goal could be partially met i.e. that LTBMC could be transduced with the human GC gene.

B.1 Construction of MFG

Subsequent studies with the N2-SV-GC vector were not encouraging that expression of the transgene was robust. In an effort to overcome the failure to achieve sustained expression with earlier vectors, we obtained the MFG vector to test its ability to express the GC gene in hematopoietic cells. The MFG retroviral vector was originally constructed by Dr. Robbins in Dr. Richard Mulligan's laboratory. Dr. Robbins provided the plasmid to us. The features of this vector are that the GC cDNA is transcribed by the retroviral LTR and the start codon of the GC cDNA was placed at the start codon of the deleted envelope protein gene. No internal promoter or dominant selectable marker is included in the construct. The structure of MFG is shown in Figure 1.

The MFG vector contains 1035 base pairs of the *gag* sequence from MMLV to increase the encapsulation of recombinant genomes in the packaging cell lines, and 350 base pairs derived from MOV-9 which contains the splice acceptor sequence and transcriptional start. An 18 base pair oligonucleotide containing NcoI and BamHI sites directly follows the MOV-9 sequence and allows for the convenient insertion of genes with compatible sites. The Nco site is positioned so that the initiation codon of the inserted gene is fused to the initiation ATG of the viral *env* gene. Thus the inserted gene is expressed from a spliced message that resembles the normal *env* message of MoMLV. The MMLV LTR controls transcription and the resulting mRNA contains the authentic 5' untranslated region of the native *gag* transcript followed directly by the open reading frame of the inserted gene.

The MFG retroviral vector was constructed in several steps. First, a three-part ligation was performed where a XhoI (position 1035; originally a NarI site) to NdeI (position 2295 in pBR322) 5'LTR fragment from a half-GAG vector, and a BamHI (originally a ClaI site at position 7675) to NdeI (position 2295 in pBR322) 3'LTR fragment from the pEM vector were ligated to a XhoI to BamHI histone H4 promoter filler fragment. Second, the NdeI site (position 2295 in pBR322) was then destroyed in the pBR322 backbone of the H4 intermediate and the XhoI site at position 1035 then NdeI linker. Third, the large NdeI to BamHI fragment from the intermediate factor was then ligated to a NdeI (position 5402) to XbaI (5764) fragment from MOV-9, containing the splice acceptor sequence, and a synthetic adapter fragment containing XbaI and BamHI overhangs (shown below) to give MFG. The synthetic adapter contains a NcoI site positioned so that the ATG within the NcoI recognition site is the initiation codon of the *env* gene. BamHI and the large fragment

Adapter sequence: CTAGACTGCCATGGCGCG
 TGACGGTACCGCGCCTAG

B.2 Construction of MFG-GC

To clone into the MFG vector, we created an Nco I site in the position of the start codon (ATG) of the GC cDNA using PCR. The sense primer (5'-CCACCATGGCTGGCAGCCTC-3') was made with a one base pair mismatch to create the Nco I site. The antisense primer (5'-GTGTACTCTCATAGCGGCTG-3') was located down stream of a Hind III site. PCR was carried out using a thermal cycler (Perkin Elmer Cetus, Norwalk, CT). The product was cut with Nco I and Hind III and a 60 bp fragment was isolated using 4% NuSieve agarose gel (Rockland, ME). On the 3' side of the GC cDNA, an Eco RI fragment of GC cDNA was isolated and the terminus was filled in with dNTP's by the Klenow fragment to create a blunt end. A Bcl I linker was ligated in the usual manner and digested with Hind III and Bcl I. The 1.7 Kb Hind III/Bcl I fragment was isolated from 1% low melting point agarose gel. The 60 bp Nco I/Hind III fragment and 1.7 Kb Hind III/Bcl I fragment were ligated to Nco I/Bam HI site in the MFG vector. The authenticity of this construct was confirmed by DNA sequencing and revealed no PCR errors or cloning artifacts. The provirus structure is shown in Figure 2. The sequence of the entire MFG-GC vector was performed by Lark Sequencing Technologies, Inc. in compliance with directives issued by the EPA/FDA. The sequencing strategy and results are included in the appendix and on disk BA.1.SEQ.

B.3 Isolation of Virus Producing Cells and Titering

In all of the following studies, we made comparisons between the MFG-GC and N2-SV-GC vectors. The MFG-GC construct was co-transfected with pSV₂Neo to the ecotropic packaging line, psi-cre, using calcium-phosphate precipitation. The molecular ratio of MFG-GC and pSV₂Neo was 20:1. After G418 selection (400ug/ml), 30 clones were isolated and grown up to near confluency in 100mm dishes. The culture medium of these clones was used as the virus source. As a target cell for titering the virus, NIH 3T3 cells were used. The virus containing medium was added to NIH 3T3 cells plated at a density of 1×10^5 cells per well in six-well dishes. Polybrene was added to the medium at concentration of 8ug/ml to facilitate uptake of the virus by cells. After 2 hour incubation at 37°C, the virus containing medium was removed and new medium was added. Following 48 hours incubation at 37°C, the cells were harvested and assayed for enzymatic activity, Western blot and Southern blot. The titer of 1×10^6 cfu/ml for the psi-cre producer of N2-SV-GC was based on the number of G418 resistant colonies of infected NIH 3T3 cells. The titer of the MFG-GC vector was estimated by Southern blots of MFG-GC infected 3T3 cells and was compared to Southern blots of non-selected 3T3 cells infected by the N2-SV-GC vector. The MFG-GC viral producer line clone (GC-psi-cre #4) with a titer of 1×10^6 was chosen for use in these experiments.

C. EX VIVO GENE TRANSFER AND SYNGENEIC BONE MARROW TRANSPLANTATION IN MICE

C.1 Gene Transfer: Mouse Hematopoietic Cells

For these studies, we followed a protocol essentially as outlined by Bodine et al⁽⁴⁰⁾. Bone marrow was obtained from animals treated for 3-5 days with 5-FU, precultured for 2 days with poke weed mitogen spleen-cell-conditioned medium and WEHI 3/b cell conditioned medium (CM), then co-cultured for 2 days with clones of VPL producing titers of 5×10^5 to 10^6 for either MFG-GC or N2-SV-GC vectors. Donor mice were male C57BL6/J-GPI^a which have a glucophosphate isomerase (GPI) isozyme designated as GPI-1^a. This serves as a convenient marker of donor cells. These donor mice also have another genetic marker known as hemoglobin "diffuse" (Hbb^d/Hbb^d). The recipient mice were young female C57BL6/J-GPI^b mice that have a GPI-1^b isoenzyme. The GPI markers permitted the estimation of the % of donor cells after transplantation.

C.2 Transduction of CFU-S

The efficiency of transduction and expression of the GC gene was estimated by analyzing individual colonies harvested from the spleen of lethally irradiated mice at 12 days after BMT. Eighty six (86) colonies from the spleens of mice given MFG-GC transduced cells and thirteen (13) colonies from mice reconstituted with marrow infected with N2-SV-GC were analyzed for comparison. Colonies from lethally irradiated mice transplanted with normal syngeneic marrow were used as controls. Representative data are shown in Figure 3. Although the Southern blot revealed that all the colonies in spleen from both groups of mice carried approximately the same number of transferred human GC genes (1-2 copies/genome) (Figure 3C), the colonies from MFG-GC mice had enzymatic activities that ranged from 2-5 fold above the controls, whereas the colonies from N2-SV-GC mice were not greater than controls (Figure 3A). Western blot results were congruous with the enzymatic activity results i.e. very little human enzyme protein could be detected in colonies derived from N2-SV-GC infected marrow (Figure 3B).

C.3 Long Term Reconstituted Mice

By two months after BMT in mice given 2×10^6 bone marrow cells, circulating white blood cells were >90% donor type as assessed by GPI, and remained >90% until sacrifice. In addition, the majority of circulating white blood cells were positive for the human gene product by immunocytochemical analysis. Animals were sacrificed between four and seven months after BMT. Various tissues from mice given MFG-GC infected cells were analyzed by enzymatic activity, Western blot, and Southern blot analyses. The enzymatic activity data in Figure 4A summarizes the measurements made on 10 animals reconstituted with MFG-GC

infected bone marrow and 2 animals reconstituted with N2-SV-GC infected bone marrow. Representative Southern analyses from three MFG-GC animals are presented in Figure 4B. Completely similar data were obtained on Southern blots of tissues from 22 animals studied with the MFG-GC vector. Southern blots of N2-SV-GC animals are shown in Figure 4C. Southern blots were positive for the human gene in all of the individual tissues. The enzymatic activity of hematopoietic tissues (bone marrow, spleen, thymus and lymph node) from mice reconstituted with MFG-GC infected marrow exceeded the activity of control tissues in all but a few specimens of lymph node and thymus. This most likely reflects the failure to obtain adequate thymus and lymph node tissue from irradiated animals. The average increase in GC activity was 3 fold in spleen and 6 fold in bone marrow. By comparison, the hematopoietic tissues from mice given N2-SV-GC infected BM cells showed little or no increase above control levels of activity. From the Southern blot analyses of hematopoietic tissues, it can be concluded that the MFG-GC vector was efficient in infecting early stem cells capable of long term reconstitution of bone marrow. The copy number was approximately 1-2/mouse genome. The data accumulated on non-hematopoietic tissues (liver, lung) was also informative. These tissues normally are supplied with bone marrow derived cells on a continuing basis. Under normal physiologic circumstances, bone marrow derived cells in these tissues are primarily macrophages. In liver, tissue macrophages (Kupffer cells) constitute approximately 15% of the organ^(83,84). If all of the liver macrophages were replaced in MFG-GC reconstituted animals by the progeny of transduced hematopoietic stem cells derived from the bone marrow, the copy number of the vector in the liver should be about 0.15/cell. The results of Southern blot analyses demonstrated that the MFG-GC vector resulted in a copy number in liver of approximately 0.1/cell. This is consistent with a high transduction efficiency of HSC by the MFG-GC vector and the ability of HSC to repopulate the macrophage lineage.

C.4 Secondary BMT

As a further measure of the ability of the MFG-GC vector to transduce self renewing pluripotent HSC, we performed secondary bone marrow transplantations using bone marrow collected from three long term reconstituted mice. These secondary transplant recipients were sacrificed at twelve days and spleen colonies were harvested. All of the 27 secondary colonies were positive for the human GC gene by Southern blot analyses (Figure 5A). The enzymatic activities of the 27 colonies were 2-7 times higher than that of control CFU-S (Figure 5B). These data provide further evidence of very efficient transduction of HSC by the bone marrow infection protocol and sustained high levels of expression of human GC in the progeny of HSC responsible for forming spleen colonies in secondary recipients.

C.5 Expression of Human GC in Differentiated Macrophages

Culture of BM with M-CSF resulted in nearly pure colonies of macrophages that were actively phagocytic as evidenced by the ability to take up latex beads (Figure 6A,B). These cultures represented approximately a five log expansion of BM precursors. The enzymatic activity of the macrophages grown from BM of eight long term reconstituted mice was on average 4 fold greater than that of control macrophages (Figure 6C). Western blots of macrophage extracts showed an intense band of human GC protein not present in control cells cultured from normal mouse bone marrow. The copy number in these cells was approximately 1-2/cell by Southern blot analyses (Figure 6D). Immunochemical staining of these cultured macrophages for the human gene product revealed that most of the cells exhibited positive staining, whereas there was no staining in control cultures (Figure 6A,B). Macrophages cultured from the bone marrow of long term reconstituted mice that were positive for the expression of the human GC gene were studied in transplantation studies. The purpose of this investigation was to determine the distribution and fate of macrophages in a syngeneic recipient. Reports published some years ago suggested that peritoneal macrophages were distributed widely, but little data was available on how long these cells persisted in the host⁽⁸⁵⁾. The availability of mouse macrophages transduced with the human gene provided marked cells which could be traced by immunocytochemical and PCR methods. In the first

experiment completed, 1×10^7 macrophages transduced with the human GC gene were injected into the tail vein of a mouse. After two weeks, the mouse was sacrificed and various tissues were analyzed by PCR using amplimers that amplified a 192 bp fragment from the provirus beginning at the Nco I site and extending into the human GC cDNA. The results are shown in Figure 7. It can be seen that the bone marrow is positive for the human gene.

C.6 Assay for the Presence of Helper Virus

Viral supernatants from the psi-cre packaging line producing MFG-GC were negative for helper virus as assessed by the BAG mobilization assay. Independently performed assays for helper virus performed by A.D. Miller were also negative for helper virus. The tissues of one of 4 control animals that were never exposed to the vector and 3 of 13 long term reconstituted animals resulted in the production of 1/2000 blue staining and Neo^r 3T3 cell targets. This experiment was repeated twice with the same result. Each of the 13 animals studied carried 1 copy of the human gene in hematopoietic tissues and expressed GC activity several fold above the background. None of the controls were positive for MFG-GC by Southern analysis. The absence of helper virus in 10 animals that highly expressed the GC gene rules out the possibility that helper assisted reinfection was related to the expression of the GC gene. The presence of an agent in control animal tissues that could be detected by the BAG assay indicates that endogenous replication competent retroviruses were present in the mouse genome. These viruses are commonly found in mice and are known to be activated by radiation^(86,87). Kaleko et. al., also have detected endogenous retroviruses in the course of gene transfer studies in mice that were unrelated to expression of the h-ADA gene in animals transplanted with retrovirally transduced bone marrow⁽⁸⁸⁾.

C.7 Localization of the Human Gene Product in the Vesicular Compartment

High resolution immunofluorescent and transmission EM ultrastructural studies were carried out by Dr. Simon Watkins in our university. These studies utilized the human specific monoclonal antibody 8E4. Control tissues from a normal mouse were completely negative. Tissues from animals reconstituted with bone marrow transduced by the MFG-GC vector showed many cells in which the human gene product could be identified. Figure 8, is a high resolution immunofluorescent stain of the bone marrow localizing the human gene product in cellular vesicles. Conspicuously absent is any staining of the nucleus. Figure 9 shows the presence of colloidal gold particles on the membranes of vesicles within the cell. The many positive figures present in these cells in vesicles, both perinuclear and beneath the plasma membrane, suggests that the cells are expressing the human GC gene product; further, that the enzyme is being distributed throughout the vesicular compartment, not only to lysosomes. These observations suggest that the enzyme is being secreted by these cells. We have demonstrated this fact in the media of cultured myoblasts transduced by the MFG-GC vector.

SUMMARY OF DATA FROM MOUSE BONE MARROW TRANSPLANTS

These data provide further evidence that retroviral vectors can efficiently transduce hematopoietic stem cells in the murine system. The presence of the human GC gene at copy numbers of 1-2 per mouse genome in primary CFU-S, in hematopoietic tissues of mice surviving up to 7 months after reconstitution, and in every spleen colony in secondary recipients demonstrates that primitive cells in the bone marrow were efficiently transduced and repopulated the tissues of the mice. It is unlikely that long lived committed progenitor cells were responsible for this effect considering the length of time after reconstitution. Comparisons to an earlier vector (N2-SV-GC) demonstrated a similar transduction efficiency in primary CFU-S and the tissues of long term reconstituted mice. This result indicates that high transduction efficiency is not a unique property of the MFG-GC vector per se, nor is it necessary in the murine system to develop a titer of greater than 10^6 particles/ml to achieve transduction efficiencies of hematopoietic stem cells that approach 100%. More importantly, this high efficiency transduction resulted from the bone

marrow culture procedures. The result was probably due to the combination of 5-FU pretreatment, extended pre-culture of the marrow with cytokines (IL-3, IL-6, and stem cell factor) in culture, as well as the coculture of the target marrow with a viral producer line of related animal origin which may elaborate other stem cell supporting cytokines.

Sustained, high expression *in vivo* of the transferred human GC gene resulted from the MFG-GC vector. The N2-SV-GC vector by comparison resulted in poor expression in spleen colonies and essentially no expression of the gene product in the tissues of long term reconstituted animals. This could have been the result of promoter suppression or down regulation of the heterologous SV40 promoter. We have not examined the reasons for this failure in detail because of the success of the MFG-GC vector. However, the difference is not only simplification of the vector. Several laboratories have observed that simplified vectors carrying the human GC gene failed to result in sustained expression of the gene *in vivo*. While expression *in vitro* or short term *in vivo* could be achieved, expression of the GC gene was rapidly lost *in vivo*. In a recent report by Correll et al, an LN vector derivative was successful in achieving sustained expression of the GC gene *in vivo*. The reasons for these differences are not clear especially since it is now known that the MFG vector is not greatly different from other retroviral vectors.

Demonstration of efficient transduction and high expression of the transferred human GC gene in the monocyte/macrophage lineage demonstrated in these studies is important to the consideration of gene therapy for GD. As discussed earlier, the macrophage is a primary target for the therapy of GD. The results provided here demonstrate that macrophage progeny derived from transduced HSC repopulate bone marrow, liver, spleen, and lung. These are the organs primarily involved in the pathology of the most common phenotype of GD. This is best appreciated by our results in liver. The copy number of the human GC gene in liver of 0.1/genome is congruous with the proportion of bone marrow derived macrophages (Kupffer cells) comprising the liver (~15%). The enzymatic activity in the livers of reconstituted animals was nearly twice the background activity indicating that macrophages carrying and expressing the human gene repopulated the organ. Direct studies of macrophages cultured from the animals demonstrated that cells of this lineage carried and efficiently expressed the human gene.

Although it is satisfying to have overcome the problem of low expression, possible problems related to overexpression of the gene product in cells might be a cause for concern when considering the potential application of gene therapy for GD. However, several lines of evidence suggest that the increase in GC expression measured in this study should not be problematic. First, the animals in these experiments appeared to be normal for up to 8 months. Animals receiving secondary transplants continued to express the human GC gene for over a year and appeared to be healthy. Furthermore, GC is a constitutively expressed house keeping enzyme that is not highly regulated. Some variation in its activity occurs normally and should be tolerated. In addition, in animal studies of enzyme replacement using macrophage targeted enzyme, the glucocerebrosidase activity of Kupffer cells was increased by more than 10 fold without harmful side effects⁽²⁶⁾. Moreover, chronic administration of 10-100mg of enzyme twice a month to more than seven hundred patients for periods of up to eight years has not resulted in any significant clinical problems.

D. TRANSDUCTION AND EXPRESSION OF THE HUMAN GC GENE IN NORMAL HUMAN AND GAUCHER PATIENT MACROPHAGES (MØ)

Human macrophages were cultured from peripheral blood from normal volunteers and patients with Gaucher disease following an approved protocol (IRB #910505). Blood was collected in 50 cc syringes containing 2.5 ml of Na heparin (1000 /ml). 5% Dextran was then added in saline solution in amount that was approximately 10% of the blood volume to a 50ml syringe, 5 ml of Dextran was added). The resulting composition was then mixed thoroughly and left undisturbed at room temperature for 40 minutes until a clean separation line was visible between the supernatant. The supernatant was layered onto Ficoll-Hypaque (Histopaque® 1077, Sigma) (F-H). The tubes were then centrifuged for 20 minutes at 530g

(1300-1500 RPM) at room temperature. A mononuclear cell (MNC) layer was easily visible as a white band in the middle of the supernatant. The supernatant above the MNC layer was discarded. The MNC layer was transferred to a separate tube for washing the Ficoll from the cells. Approximately 20-40 ml of Dulbecco's phosphate buffered saline (D-PBS) (Gibco) (1x) was added to the tubes containing the MNC's, or alternatively, KRP-glucose could be used. The cells were washed thoroughly at least two times, and the pellets were resuspended in culture medium (1640 RPMI (Gibco), 10% FBS, 10% human albumin). The cells were then counted and a viability assay was performed using trypan blue exclusion. Cytospin slides for Wright stain were prepared and the percentages of resultant isolated MØ and lymphocytes were counted. In order to culture macrophages, the mononuclear cells from the preceding step were used. The cell count was adjusted to $\sim 5 \times 10^6$ cells/ml with culture medium (1640 RPMI, 10% FBS, 10% human albumin), and the cells were plated in 35mm culture dishes (approximately 10 cm² surface) so that the plating density was between $0.2-0.6 \times 10^6$ MØ/cm². The cells were then incubated at 37°C in 5% CO₂ for 60 minutes, and non-adherent cells were removed after the incubation. Then 1 ml of D-PBS (1x) was gently placed in the dish, and the dish was shaken to remove non-adherent cells left in the bottom of the dish. 2ml of previously warmed culture media was then added to each 35 mm dish, and the cells were then incubated at 37°C in 5% CO₂. The culture medium should be changed only once a week. In order to infect human macrophages in culture with MFG-GC, the culture medium from the cultured macrophages of the previous step was removed from each culture dish and 1 ml of viral supernatant obtained from the amphotropic producer cell line (titer: 5×10^5) was added to each culture dish along with Polybrene (8 ug/ml). The dishes were then incubated at 37°C in 5% CO₂ for an additional 48-72 hours. The plates were checked to assure an adequate number of adherent cells were present. The culture medium containing the viral supernatant was removed from each culture dish, and each plate was washed with D-PBS (1x), and the D-PBS was removed. Then 1 ml of lysis buffer (0.05M K-P, 1% Triton X-100, pH 6.5) was added to each plate, and a rubber policeman was used to detach all cells from the bottoms of each dish. The cell lysates were transferred to small Eppendorf tubes and placed on ice. The lysates were sonicated and processed for GC enzymatic activity and protein assays as described previously. As shown in Figure 10, the enzymatic activity assays of these cells demonstrated that the MFG-GC vector is able to impart approximately 75 U/mg cell protein to either normal macrophages or the macrophages from patients. This increment of enzyme corrects the enzymatic deficiency completely in the cells of patients with Gaucher disease.

E. GENE TRANSFER AND EXPRESSION IN CD34⁺ CELLS FROM BLOOD

CD34⁺ cells contain a population of pluripotent stem cells capable of reconstituting the bone marrow in primates and man. Recent studies by several investigators have shown that the human peripheral blood CD34⁺ cell (PBSC) can be efficiently transduced by retroviral vectors. Because of the ability to concentrate these cells in a small volume, the logistics of ex vivo transduction become greatly simplified. Furthermore, the ratio of virus to stem cell in infection protocols can be enhanced. This could lead to more efficient transduction. Because of this economy and because the bone marrow of patients with Gaucher disease are frequently "packed" (full of cellular material and scar; they frequently can not be aspirated), the PBSC are particularly ideal cells for gene transfer/therapy studies. The methods used for isolation and flow cytometric analysis of CD34⁺ cells are included in the appendix. The results of a typical >10 fold enrichment with 87.6% purity are shown in Figure 11. In these laboratory scale columns, enrichment is less than in the large clinical scale columns (see appendix). Clonogenic assays reveal an increase in CFU-GM of about 3 fold (Figure 12).

Results of our initial experiments with CD34⁺ enriched PB cells from G-CSF primed lymphoma patients show that transduction occurs and leads to expression of the glucocerebrosidase gene in these cells and their progeny using the MFG-GC retroviral vector. In Figure 13 are shown enzyme activities of cells harvested shortly after infection in three different mixtures of cytokines: 1) IL-3, IL-6, SCF, and GM-CSF; 2) IL-3, IL-6, and SCF;

and 3) SCF and PIXY. In each case the enzyme activity in the MFG-GC infected group was more than 80% higher than the level of endogenous activity in noninfected cells grown under the same conditions. Control samples infected with a retroviral vector, containing the bacterial *lacZ* gene, did not differ significantly from noninfected cells, eliminating the possibility that increased activity from MFG-GC transduced cells was a result of nonspecific viral exposure or infection conditions. Infected cells were expanded *in vitro* for two weeks following infection, and a sustained elevation was observed in enzyme activity in the experimental group versus control cells (Figure 14). Southern blot hybridization of DNA from these cells suggested a transduction efficiency of 5 to 15%.

In two subsequent experiments (Experiments #2 and #3), significantly increased GC expression was not observed following infection procedures. In these experiments, rat recombinant SCF (rrSCF) was used during infection and expansion of the cells, and although the biological activity of this factor was expected to be similar to that of human recombinant SCF (hrSCF) (Langly, et al, 1992)^(E3), it became clear in Experiment #4 that the cytokine activity was suboptimal using rrSCF from this particular source (Figure 15). In the latter experiment (#4), cells were harvested at the point at which a decline in growth rate was observed. Analysis of glucocerebrosidase activity in these cells indicated that elevation of activity had occurred in each of several different groups following infection in comparison to similarly cultured noninfected cells (Figure 16). These cells had been infected under a variety of conditions in an effort to uncover a reason for the lack of response in the two previous experiments. Two mixtures of cytokines were used; 1) IL-3, IL-6 and SCF, or 2) PIXY, IL-6 and SCF. The latter group was further subdivided into cells infected in the presence of a stromal cell layer, in the presence of human serum, on Petri dishes and on treated tissue culture dishes. The specific activity of these samples was lower across all the groups than in previous experiments (control cells ~150 U/mg vs >200 U/mg), perhaps as a result of the declining viability of these cells at the time, but the elevation for infected cells was about 70% above endogenous levels.

In Experiment #5, for which hrSCF was used throughout infection and expansion phases, the specific activity was once again >200 U/mg, an increase of more than 100% above endogenous GC activity (Figure 17). Southern blot hybridization suggests a transduction efficiency of >20% (Figure 18). In subsequent experiments, we have demonstrated that normal CD34⁺ cells are transduced and express 2-4 times the activity of GC (Figure 19). We also have studied some of the variables that affect the transduction efficiency and resultant expression of GC in CD34⁺ cells. From current results, we have concluded that the combination of IL-3, IL-6 and SCF provides the most consistent results. Further, there is a linear relationship between total number of doses of viral supernatants and GC activity expressed in CD34⁺ cells (Figure 20). Using these conditions, CD34⁺ cells obtained from Gaucher bone marrow have shown an increase in GC activity of 20-40 fold essentially equalling the results in transduced CD34⁺ cells from controls (Figure 21). These cells kept in culture for 3 weeks maintain the elevated levels of glucocerebrosidase activity.

We have recently demonstrated that Gaucher CD34⁺ cells can be rapidly evaluated for transduction efficiency using a conventional chromogenic immunocytochemical assay for glucocerebrosidase. Using this procedure, CD34⁺ cells from a Gaucher patient give no detectable color formation, whereas, many cells in the infected population gave a strong signal (see Figure 30). Counting ten high power fields and scoring for immunocytochemical positive cells, an estimated transduction efficiency of approximately 20% can be calculated. The advantage of this method is its speed. The analysis can be done within one day. We will utilize this method in characterizing cells to be reintroduced to the patients in the study (see Section V. I).

The colony forming efficiency in methylcellulose has been determined for freshly enriched CD34⁺ cells and for infected and control cells following infection procedures. Results from 3 experiments indicated that between 30-100% of the colony forming efficiency remains after infection.

V. EXPERIMENTAL DESIGN

A. OVERVIEW

The hypothesis of this study is that autologous bone marrow transplantation of genetically corrected peripheral blood stem cells (PBSC) will result in a sustained reversal of the phenotype in patients with Gaucher disease. The specific aims to be achieved are:

- 1) Transfer of the human GC gene into PBSC ($CD34^+$) obtained from patients with Gaucher disease.
- 2) Transplantation of transduced PBSC autologously to patients.
- 3) Estimation of engraftment of $CD34^+$ cells by measurement of the carriage and expression of the transferred gene and its duration in peripheral blood leukocytes (PBL).
- 4) Assessment of the clinical effects of transplanting genetically corrected PBSC in patients with Gaucher disease.

This study is a clinical trial of gene therapy for patients with Gaucher disease. It involves the use of G-CSF to stimulate the release of $CD34^+$ cells into the blood from the bone marrow. These cells are harvested by leukopheresis, density gradient centrifugation, and percolation over an immunoaffinity resin (CEPRATE™SC) to harvest $CD34^+$ cells. The cell fraction contains 50-80% $CD34^+$ cells and is enriched for cells capable of long-term reconstitution of the bone marrow (stem cells).

Peripheral blood $CD34^+$ cells enriched for stem cells (PBSC) will be transduced with the normal GC gene by exposure to supernatants from the amphotrophic producer lines of the vector once a day for four days. The PBSC will be collected and washed. An aliquot of the harvest will be immediately lysed and assayed for glucocerebrosidase activity. Only harvests with GC activities within the range of normal will be used in transplantation studies. Cells also will be assessed for carriage of the GC gene by Southern analysis and will be tested for contaminating materials. Cells will be viably frozen until the results of tests are complete.

After obtaining negative results in a routine sterility test and negative tests for helper virus in the supernatant of the *ex vivo* transduction mixture, patients with Gaucher disease will be transplanted with $2 \times 10^6/\text{kg}$ PBSC. This is the dose that has become standard as a reconstituting dosage of PBSC. In this study we will evaluate whether patients will require myeloablation to achieve a successful result. The decision to use myeloablative preparation will be made on the basis of correction of enzyme deficiency in the leukocytes of patients transplanted with genetically corrected PBSC who have not received myeloablative preparation. Results in the first two patients studied will be used to make this decision. Patients usually engraft rapidly with PBSC and could be considered to be complete within one month. Therefore, assay of PBL for GC activity will be performed after that interval of time. The assays of enzyme activity will be used to make the decision. The results in these first two patients will determine the approach to be used in all subsequent patients studied. If the amount of GC in leukocytes is not increased 2 fold above the deficient level, it will be concluded that an inadequate number of corrected stem cells have engrafted. If this is the case, patients will be prepared with cyclophosphamide ($8\text{g}/\text{m}^2$) to partially ablate the genetically defective marrow. This preparation should permit replacement of at least part of the bone marrow with genetically corrected cells. The approach of partial marrow ablation has been chosen to reduce the risks to the patient.

After having established the restoration of enzymatic activity and carriage of the transferred gene, patients will be studied for the clinical responses to the therapy. This includes repeated measurements of clinical and laboratory parameters known to be abnormal

in patients with Gaucher disease. The measurements to be made monthly are physical exam, white count, hemoglobin, platelet count, serum non-tartrate inhibitable acid phosphatase, and serum angiotensin converting enzyme. Measurements to be made every six months are MRI of liver and spleen for size, x-ray films and scans of long bones and pelvis, and biopsy of the bone marrow. An overview of the study is diagramed in Figure 22.

B. SELECTION OF STUDY CANDIDATES

Patients with an enzyme assay proven diagnosis of Gaucher disease will be recruited from clinics at the University of Pittsburgh and from referrals. They will be informed of the risks and inconveniences of the study by Dr. Barranger. They will be required to sign a consent form. Either of two types of patients will be selected for study. One type of patient is the enzyme treated patient, the other is the patient with evident disease not currently treated with enzyme. In the enzyme treated patient, the criterion of response to gene therapy is the ability to reduce the dosage of glucocerebrosidase without clinical deterioration as assessed by clinical parameters. Therefore, to be eligible for the study, these enzyme treated patients 1) must have had significant signs and symptoms of Gaucher disease prior to the initiation of therapy with glucocerebrosidase enzyme infusions and 2) have responded to treatment in a measurable way. The specific criteria of improvement in response to enzyme are:

- 1) An increase in hemoglobin of 2gm/dl
- 2) An increase in platelet count of 50%
- 3) A decrease in spleen size of at least 25% or a decrease in liver size of at least 25%
- 4) An improvement in MRI or X-ray of the bones
- 5) A 50% decrease in non-tartrate inhibitable acid phosphatase
- 6) A 50% decrease in angiotensin converting enzyme

The patient would be judged to have a positive response to gene therapy if these improvements are maintained for 1 year following the cessation of enzyme therapy. The schedule of planned enzyme dosage reduction is 50% at the start of the study, an additional 50% reduction at three month intervals times three, then discontinuation of enzyme. If the patient deteriorates as assessed by clinical exam and laboratory studies, enzyme therapy will be reinstituted. Since patients change slowly over a period of months after enzyme withdrawal, this plan should be safe. The second type of patient eligible for the study is the previously untreated patient with evident disease, but in whom immediate enzyme therapy would not be life-saving. These patients will have measurable clinical signs and symptoms, but would not die from their disease within two years if not treated with enzyme. To be eligible for the study, these candidates must have two of the following signs or symptoms:

- 1) Splenomegaly or Hepatomegaly
- 2) Anemia with a hemoglobin < 11g/dl
- 3) Thrombocytopenia with a platelet count < 90,000
- 4) Disabling bone pain with degenerative changes on X-ray films
- 5) Pulmonary compromise with clubbing and hypoxemia ($PaO_2 < 70$)
- 6) Biopsy proven cirrhosis and elevation of hepatic parenchymal enzymes
- 7) Esophageal variceal bleeding

Ineligibility

No patient is eligible to participate in the study without a proven deficiency of glucocerebrosidase. Each patient must have clinical evidence of significant disease as described above. To participate, a patient must be able to tolerate physical examination, blood drawing not to exceed 5% of blood volume, x-rays, nuclear magnetic resonance imaging and electrocardiography. No patient with malignancy, positive HIV serology or cardiorespiratory instability will be accepted into the study. Pregnant women will not be accepted into the study. Women will be advised not to become pregnant during the study.

Stopping Rule

If at any time during the study, the presence of helper virus is detected, the study will be halted until all materials are certified to be helper free. Transplanted patients will be monitored for retroviremia with helper virus by testing the serum and white blood cells from 50ml of blood by the BAG assay. This monitoring will be repeated every 30 days. If there is evidence of retroviremia, the patient will be treated with antibodies to helper virus. The study will be halted until the source of the retroviremia is determined and corrected. If other studies of gene transfer using retroviral vectors indicate a reason for stopping this study, this will be done.

Post Mortem Examination

It is very unlikely that a patient will die during their participation in this protocol. Should death occur, a post mortem examination will be requested.

C. DESCRIPTION AND CHARACTERIZATION OF THE VECTOR FOR GENE TRANSFER

The MFG-GC vector was used for the preclinical studies described in Sections IV: C,D and E. This vector contains 1035 bases of the gag gene in an open reading frame. We have concluded that the MFG vector could be improved for human trials by eliminating the in frame gag sequences, thus rendering the vector unable to synthesize truncated gag-related peptides of any significant size as well as decreasing the possibility of replication competent retrovirus (RCR). We accomplished this by performing partial SmaI digestion of the vector containing the GC cDNA and inserting an eight base pair Sac II linker 3' to Hae III site at the ATG of the half-gag gene in the retroviral sequence. This creates an additional mutation in the vector and destroys the open reading frame for the gag polypeptide sequence. We confirmed the insertion of the Sac II linker by sequence analysis across the region of the Sma-I site. This sequencing was duplicated by Lark Sequencing Technologies who obtained identical results. The sequence analysis is enclosed on disks labeled BA123.SEQ and shown in Figure 23.

The new vector, called R-GC, was co-transfected to the GP+E86 packaging line. Stable clones were isolated and screened for the activity of glucocerebrosidase. We have found this identification process very useful in selecting clones to be carried on to high titer viral producing lines (VPL). The GP+E86 transfected cell line was used to generate a viral supernatant. This supernatant was used to infect the psi-crip and GP+envAM12 packaging lines. The activity of the human enzyme in these cells is shown in Figure 24. The significant increases in the activity of human GC in cells transduced by R-GC demonstrates the ability of the vector to transmit the human GC gene and express the gene product. Studies are in progress to select clones of the best VPL's which will be compared to MFG-GC producers in parallel studies of titer. Transduction efficiency and expression in human Gaucher CD34⁺ cells will be compared to results with MFG-GC. The best VPL will be characterized and studied for the presence of contaminating materials, especially helper virus. This will be discussed in detail in the following paragraphs.

D. PRODUCTION AND CHARACTERIZATION OF THE VIRAL PRODUCER LINES

The following methods were used to develop the MFG-GC viral producer lines. In the initial studies with ecotropic producers of MFG, we made comparisons between the MFG-GC and N2-SV-GC vectors. The MFG-GC construct was co-transfected with pSV₂Neo to the ecotropic packaging line, Psi-cre, using calcium phosphate precipitation. The molecular ratio of MFG-GC and pSV₂Neo was 20:1. After G418 selection (400ug/ml), 30 clones were isolated and grown up to near confluency in 100 mm dishes. The culture medium of these clones was used as the virus source. As a target cell for titering the virus NIH 3T3 cells were plated at a density of 1×10^5 cells per well in six-well dishes. Polybrene was added to the medium at a concentration of 8 ug/ml to facilitate uptake of the virus by cells. After 2 hour incubation at 37°C, the virus-containing medium was removed and new medium was added. Following 48 hours incubation at 37°C, the cells were harvested and assayed for enzymatic

activity, Western blot and Southern blot. The titer of 1×10^6 cfu/ml for the Psi-cre producer of N2-SV-GC was based on the number of G418 resistant colonies of infected NIH 3T3 cells. The titer of the MFG-GC vector was estimated by Southern blots of non-selected 3T3 cells infected by the N2-SV-GC vector. The MFG-GC viral producer line clone (GC-Psi-cre #4) with a titer of 1×10^6 was chosen for use in the experiments described in Sections IV: C, D and E.

The amphotropic producer line of MFG-GC was developed by cross infection of the Psi-crip amphotropic packaging cell line with viral supernatants from the ecotropic GC-Psi-cre #4 producer. Multiply infected (4x) Psi-crip cells were plated at limiting dilution in 96 well plates. Of twenty-two clones, twelve were expanded and were assayed for enzyme activity. Supernatants were collected and assayed for transduction efficiency based upon enzyme activity in 3T3 targets. A single clone, cc-2, yielded superior supernatants and was chosen for quantity production (Figure 25). This producer exhibited slow cell growth and contact inhibition, at the same time yielding consistently high titer supernatant. From one batch of confluent cells, supernatants were collected every day for almost three weeks without trypsinization and without measurable decline in viral production (Figure 26).

The cc-2 producer was titered as follows. Target 3T3 cells were transduced with varying volumes of a representative supernatant. The infected cells were harvested, and DNA was extracted and analyzed by Southern blot hybridization using a probe for GC. A Beta scanner was used to quantitate the amount of the vector fragment for each target cell DNA sample. These radioactivities were compared to the radioactivity from DNA prepared from a cloned cell line containing a known number of copies (3 copies) of the vector sequence. (The copy number of this infected clone was determined on a separate blot using EcoRI restriction to produce a unique band for each integration site. An identical 3-band pattern was observed in 5/5 subclones.) A linear relationship was observed between copy number in the target 3T3 cells and amount of supernatant used for infection (Figure 27). The average copy number of the target cells multiplied times the number of target cells present during infection therefore provides a minimum estimate of the number of virus particles present in the volume of supernatant used. The linearity of the relationship between volume of supernatant and average copy number supports the validity of this approach for estimation of titer. Since an average copy number of 35 was observed for infection of 3×10^5 cells with 1 ml of supernatant, the titer of the supernatant was at least $35 \times 3 \times 10^5/\text{ml} = 10^7$ infectious virus particles/ml.

The absence of helper virus was verified by coculture of the producer cc-2 cells with BAG infected 3T3 indicator cells. Cocultures were split 4 times in the presence of 8ug/ml polybrene over a period of three weeks. Supernatants prepared from these cocultures were used at full strength to infect 3T3 target cells. The supernatants from BAG/cc-2 cocultures were negative for producing Xgal staining cells or for G418^R cells among infected targets. Control cultures of BAG/3T3 cells with pA317 producer cells, from which helper activity had been induced by previous coculture with an ecotropic packaging line, were positive in this series of assays. This assay is extremely sensitive for detection of helper virus, particularly when multiple passages over time in the presence of polybrene are incorporated.

The viral producer line of R-GC will be developed by transfection of the psi-crip packaging line and selection of high producers as outlined above. Neither ping-pong infection nor cross-infection will be used in developing the R-GC vector. The VPL of R-GC will be characterized as to its ability to reproducibly produce a high titer viral supernatant. The titering methods have been described in earlier sections of this application. The selected VPL will be placed in cultures and expanded to provide at least 25 cryovials. The VPL will be brought into culture and a minimum of 100ml of viral supernatant produced and tested for titer. This test will be repeated on separate mini-production runs. Our experience with different producers is that the cell line remains stable for many months. Tests will also be conducted to assess the stability of the frozen viral supernatant. Again, our experience is that the supernatant is stable for many months.

We will document these characteristics for the VPL of R-GC chosen to be used to establish the master cell bank. These stability tests will be carried out in the principal investigator's laboratory. In addition, the VPL will be evaluated for its ability to produce a viral supernatant that can correct the enzyme deficiency of GC in CD34⁺ cells obtained from Gaucher patient bone marrow. We are fortunate to have a large supply of patient marrow obtained during a knee replacement. This otherwise discarded material is very useful in evaluating vector quality, stability, and determining the optimum conditions for infection as described earlier.

Once we have selected and characterized the best producer line, we will test it for contaminating materials according to the guidelines published by the FDA and recommendations communicated to the RAC. Because of recent concerns about RCR, we will incorporate sensitive tests for the presence of helper virus. We expect this testing to be complete by mid summer 1993 due in part to the near term completion of the Human Gene Therapy Applications Laboratory (HGTAL) where we will perform some of the testing and production of the supernatants. Testing, to be performed by a contract laboratory, will include the following to be performed on the master bank (MCB) of the VPL:

Sterility	Cell culture ID
Isozyme and Cytogenetic Analysis	Mycoplasma
Extended XC Plaque	Extended S+/L- Focus
MAP Antibody Production	In-Vivo Virus
In-Vitro Virus	Co-Cultivation with RD Cells
Bovine Viruses	Porcine Parvovirus

In addition, the supernatant of the MCB will be amplified in 3T3 cells and the supernatants exposed to PG-4 (feline) cells and re-tested by the S+/L- focus assay. As a measure and an approximation of the clinical application, the MCB will be used to generate a viral supernatant with a titer of 10⁷. This supernatant will be used in a study that maintains human CD34⁺ cells in culture for 7 days. The human cells will be exposed to viral supernatant produced by the MCB at an MOI of 10 virus particles/CD34⁺ cell. Repeated infections of the cell will take place at a frequency of 1/day for 4 days beginning on day 3 of the culture. The culture supernatants collected during media refreshments and the final supernatant will be pooled and tested using the BAG mobilization assay and simultaneously submitted to a contract lab for S+/L- focus assay with the PG-4 cell line after amplification in 3T3 cells. Copies of the protocols for the testing done by the contract laboratory are enclosed in the appendix.

E. PRODUCTION AND CHARACTERIZATION OF THE VIRAL SUPERNATANT

Each patient will require 2 x 10⁶ CD34⁺ cell/kg. This is the dose used to reconstitute the bone marrow in patients who have been myeloablated. The dose needed to result in engraftment in an unprepared recipient is unknown. Studies of syngeneic bone marrow transplants in mice of the opposite sex and autologous transplants in dogs using retrovirally transduced marrow suggest that 1-10% of the donor cells can stably engraft in the host animal without preparative myeloablation^(67,94). These results provide at least the possibility that some niche exists for autologously transplanted bone marrow cells. Our proposed study will evaluate this possibility in the Gaucher patient. We have selected a dose of CD34⁺ cells that has been used to reconstitute the bone marrow in myeloablated cancer patients. At this dose of cells and using an MOI of 10/1 with daily exposures for 4 consecutive days, approximately 8 x 10⁹ virus particles will be required for each patient in the study. At a titer of 10⁷ pfu/ml, 800ml would be sufficient to transduce the CD34⁺ cells from a patient (~1.4 x 10⁸ cells). In the preparation of the supernatant, we plan to produce 5 liters from the producer line MCB (See appendix for the protocol on raising supernatants). We will use this production run for testing the titer and confirming the transduction efficiency of the lot and its ability to express the GC gene in our stock of CD34⁺ cells obtained from bone marrow of a Gaucher patient. This production run will also

be used in tests of its safety including sterility and tests for the presence RCR. These tests will include the BAG mobilization assay done in our own laboratory and the extended S+/L- focus assay performed by our contractor in PG-4 feline cells using 5% of the production run after amplification in 3T3 cells.

In an effort to try to evaluate the occurrence of RCR during an extended period of culture, we will attempt to extend the culture period to three weeks in a post production run. This step should yield another 5 liters of supernatant. We have been successful on a pilot scale with the cc-2 producer to achieve this goal. Again, 5% of the post-production run will be assayed for RCR by amplification in 3T3 cells and performance of the S+/L- extended focus assay in PG-4 cells.

F. G-CSF PRIMING AND COLLECTION OF A MONONUCLEAR CELL FRACTION

G-CSF mobilization will be used in patients with Gaucher disease to increase the number of PBSC in the blood. Candidates will receive G-CSF at a dosage of 5 mcg/kg/day by subcutaneous injection on consecutive afternoons. Injections must be scheduled to begin on a Wednesday, in order for leukopheresis and laboratory processing to be performed on consecutive weekdays of the following week. Daily morning WBC count and differential counts will be obtained, including weekends. Daily monitoring is essential, since WBC can rise rapidly on G-CSF. If WBC count is $\geq 45,000/\text{ml}$, G-CSF dose is to be reduced to 3 mcg/kg/day. If WBC count is $\geq 75,000/\text{ml}$, G-CSF is to be discontinued.

Leukopheresis procedures will start on day 6 (Monday), to be continued on a daily morning schedule until a total mononuclear cell (MNC) yield of $7 \times 10^8/\text{kg}$ is obtained. It is anticipated that 3-5 collections (i.e., Monday through Wednesday, or Monday through Friday) will be required for the majority of patients to achieve this yield. If a sufficient number of MNC is not collected by day 10 (Friday), daily G-CSF may be continued at the discretion of the patient's hematologist/oncologist and the Principal Investigator, with leukopheresis resuming on day 13, until an adequate MNC dose is obtained. If cytopenia ($\text{WBC} < 3,000/\text{ul}$ or platelets $< 50,000$) develops during, or as a result of leukopheresis, the procedure will be postponed until recovery. G-CSF will be continued through this interval.

Seven to ten liters of the patient's blood will be processed, per procedure, depending on patient's total blood volume and hematocrit. Samples from each mononuclear cell product will be obtained for hemoglobin, hematocrit, total WBC and differential, platelet count; colony assays; flow cytometry; and microbiology. Each mononuclear cell product will be enriched for CD34^+ cells on the day of collection.

G. ENRICHMENT OF CD34^+ CELLS

The mononuclear cell (MNC) fraction will be enriched using the Cephate™SC Stem Cell Concentrator. A copy of the details of the procedure are included in the appendix. In brief, the MNC are labeled with the 12.8 biotinylated anti -CD34 antibody and loaded onto a CellPro column which permits separation of the CD34^+ cells. Typical enrichment is 40-50 fold and the cells are usually more than 80% pure by FACS analysis (See appendix). At the end of the separation, the CD34^+ cells will be viably frozen as described in the protocol.

H. TRANSDUCTION OF CD34^+ CELLS

The CD34^+ cells obtained are transduced according to the procedure outlined below.

- 1) CD34^+ cells from peripheral blood are suspended in medium containing a mixture of cytokines. The mixture consists of rhSCF (10ng/ml), rhIL-6 (10ng/ml), and rhIL-3 (10ng/ml) in complete LTBM. The cells are plated into 10 cm dishes at an approximate concentration of 2×10^6 . The cells are incubated for 6 days.
- 2) For infection, the viral supernatant is added to the dish of cells, once or twice daily. Protamine sulfate is added to yield a final concentration of 4 ug/ml. The volume of supe added will be approximately 2 ml with 5 ml of media.

- 3) On the following day, the medium is removed and stored; the cells are transferred to a centrifuge tube, pelleted at 500g for 15 minutes, resuspended in fresh medium with cytokines, and returned to the dish.
- 4) The infection cycle is repeated as in step 2 for a total 4 days.
- 5) The growth of the cells is monitored by periodically counting suspended cells using a hemacytometer.
- 6) The cells are pelleted by centrifugation at 500g for 15 minutes. The pellet is then washed twice in cold PBS and resuspended at a cell concentration of $1 \times 10^6/\text{ml}$ and viably frozen.

I. MEASUREMENT OF TRANSDUCTION EFFICIENCY AND EXPRESSION OF THE TRANSGENE

The infected CD34⁺ cells will be tested for transmission of the human GC gene by the R-GC vector using the immunocytochemical method for GC as described in section V.3 and by Southern analysis as well as by analysis of GC enzymatic activity in the cell pool (see below). Immediately after harvest, the CD34⁺ cells will be viably frozen according to the protocol provided in the appendix for the separation of CD34⁺ cells. We have experience with multiple preparations, that subsequently thawed cells are 80% viable. This characteristic permits us to evaluate the efficiency of gene transfer and expression in CD34⁺ cells during the 3 week interval when the supernatants are being tested for bacterial and viral contamination (described below). For characterization of the efficiency of gene transfer, one 10cm plate of CD34⁺ cells (2×10^6) at the end of infection will be expanded for 5 days. This will yield $\sim 10^7$ cells. These cells will be assayed for transduction efficiency and expression of the transgene. We have selected a minimal transduction efficiency of 20% as acceptable because this has resulted in average incremented activity of the target cell pool equal to 2-4 times the activity of controls (20-40 times the activity of Gaucher patient cells) (see Section IV.E). We will accept the cells if the enzymatic activity of the pool is within the range of 100-500% of control cells and 20% of the cells stain for the transgene product.

J. TESTING OF FINAL PRODUCT FOR CONTAMINATION BACTERIAL AND VIRAL CONTAMINATION

An aliquot of the CD34⁺ cell mixture at the end of the infection will be tested for viability using eosin exclusion. A sample will be submitted to our contract laboratory for gram stain and culture for bacterial and fungal contaminants, mycoplasma screen and a pyrogenicity test. An evaluation of contamination by replication competent retrovirus (RCR) will be made by testing the pooled supernatants from the transduction step. An aliquot of 100 ml, representing more than 10% of the supernatant, will be amplified on 3T3 cells and the resulting supernatant tested in an S+/L- focus assay on PG-4 cells. We will also perform a BAG mobilization assay in our own laboratory. Failure to pass any of these tests will preclude use of the transduced cells in a patient.

K. INFUSION OF TRANSDUCED CELLS

Frozen CD34⁺ cells will be thawed using a slow dilution method to provide optimum viability and recovery of frozen cells. If necessary, concentration of progenitor cells will be accomplished using the CEPRATE™ SC Stem Cell Concentrator, as described in the appendix. An IND will be obtained for the use of this device in this protocol. The dose of CD34⁺ cells will be $2 \times 10^6/\text{kg}$.

On the morning of infusion, the following data will be obtained and recorded.

- Brief medical history and interim clinical status
- Complete physical examination including temperature, pulse, respiratory rate and blood pressure
- Laboratory studies: CBC, differential, platelet count, BUN, creatinine, SGOT SGPT, alkaline phosphatase, bilirubin and urinalysis. Marrow will be thawed as outlined and infused through the patient's central venous catheter.

Epinephrine and antihistamines will be available at the bedside on a resuscitation cart as a precaution. Patients will be treated with benadryl (25mg) and Tylenol (625mg) 30 minutes prior to PSC infusion. Temperature, pulse, respiratory rate and blood pressure will be monitored every 15 minutes. Symptoms and signs of infusional toxicity will be recorded.

L. ESTIMATION OF ENGRAFTMENT

The first two patients receiving genetically corrected CD34⁺ cells will not receive myeloablation prior to treatment. As mentioned earlier in this application, some data suggests that engraftment can occur using whole bone marrow in an unprepared host⁽¹⁾. These data provide some support for the concept that a habitable environment (niche) exists in which hematopoietic stem cells can engraft. While this concept remains controversial, it deserves testing in man because of the potentially reduced risks afforded by this approach. It would be to the patient's advantage to be able to autologously transplant genetically corrected cells without myeloablation. Therefore, we feel obligated to evaluate this possibility. Furthermore, enrichment for stem cells by CD34 selection increases the chance of engraftment of cells with the potential of bone marrow reconstitution on a long term basis. Enrichment for CD34⁺ cells may provide a greater chance of success than earlier studies using whole bone marrow. This favorably increases the odds of establishing permanent fraction of bone marrow that is genetically corrected and able to produce enzymatically competent macrophages. A critical appraisal to be made is whether a fractional correction of the marrow with genetically and enzymatically repaired cells has a chance of being therapeutic. Animal and human studies suggest that this is possible. In studies of the gus/gus model of mucopolysaccharidosis VII (glucocerebrosidase deficiency), bone marrow transplantation with genetically corrected whole marrow, in which expression of the transgene was suboptimal, resulted in enzymatic correction of tissues to only 2-5% of control levels⁽⁸⁹⁾. Despite this, the visceral manifestations of the disease were dramatically reversed. Furthermore, in Gaucher disease and Pompe disease, orthotopic liver transplantation results in a small fractional chimerization of the recipient marrow by donor marrow. Chimerization is believed to be a contributing factor to the therapeutic response in these disorders⁽⁹⁰⁾. Furthermore, targeting of glucocerebrosidase to macrophages results in only a temporary and incomplete correction of enzymatically competent cells, yet is therapeutic. From available data on the genetic correction of the LLD receptor in the Watanabe rabbit, it would also appear that a partial correction of functional gene product is sufficient to result in a significant reversal of phenotype⁽⁹¹⁾. These collective experiences suggest that a small correction of functional enzymatic activity in GD will result in a therapeutic response.

The protocol is designed to evaluate the possibility that engraftment of genetically corrected CD34⁺ cells will result in a biochemical and clinical response without myeloablation before proceeding to a regimen of partial myeloablation. (See Assessment of Risk/Benefit for a detailed discussion). The assessment of engraftment in these steps of the protocol depends on the estimation of enzymatic activity and immunocytochemical staining in peripheral blood leukocytes and bone marrow and on the measurement of indicators of therapeutic response in two clinical laboratory values in patients with Gaucher disease who have had a therapeutic response to enzyme replacement (see below).

In mice, we have routinely observed an increase of 4-5 times the endogenous activity of glucocerebrosidase in freshly transduced donor marrow, primary CFU-S, secondary CFU-S, hematopoietic tissues from long-term reconstituted mice, and macrophages cultured from long-term reconstituted mice. In CD34⁺ cells from normal individuals, we have observed an increase in specific activity of GC of 2-4 fold. The transduction efficiency in these targets was 10-20% or 0.1-0.2 transgene/genome. In CD34⁺ cells from Gaucher patients, the increment of activity is approximately 10 times greater owing to the fact that the endogenous activity of these enzyme deficient cells is approximately 10% of the control. Immunocytochemical staining of the patient cells is negative. Transduced cells show

strongly positive staining. These results indicate that the expression of the transgene is independent of the genetic deficiency of the target cell. This fact is both satisfying and reassuring that other genetic or epigenetic factors are not relevant to the expression of the transgene in the cells of Gaucher patients. We forecast that the detection of engraftment will be feasible using GC enzymatic activity as the indicator based on the following calculations.

Starting Specific Activity (SA) of Gaucher PBL	Estimated Specific Activity of Corrected PBL (4 X Control)	% Genetically Corrected PBL (% Engraftment)	Expected SA of PBL	% Increase
1.0 Unit/mg	40 Units/mg	0	1.0	0
1.0 Unit/mg	40 Units/mg	1	1.39	30
1.0 Unit/mg	40 Units/mg	5	2.99	300
1.0 Unit/mg	40 Units/mg	10	4.99	500

We do not expect a selective advantage of genetically corrected stem cells. However, theoretically, enzymatically competent tissue macrophages produced by the engrafted stem cells may function to degrade existing glucosylceramide stores. This has been shown in patients receiving enzyme therapy. Thus, we plan to measure angiotensin converting enzyme and non-tartrate inhibitable acid phosphatase in the plasma of autologously transplanted patients. Both of these measures are markers of macrophage storage of lipid and are indicators of a therapeutic response. We have shown them to rapidly decline in patients treated with enzyme replacement at optimal and sub-optimal doses (Figures 28-29). While we do not expect Southern analysis to be sensitive enough to detect low percentages of engraftment, we will perform these analyses as well. Samples will also be preserved for semi-quantitative PCR analyses of the human GC in the R-GC vector in subsequent studies.

If studies of PBL enzymatic activity and indicators of MØ storage do not indicate sufficient engraftment, we will proceed to partially ablate the marrow of patients using cytoxan alone. The dose will be 7g/m². This dose has been used to stimulate the release of progenitors and CD34⁺ cells to the blood stream by the partial destruction of marrow. This stimulates the cycling of the marrow and increases the proportion of stem cells in the blood. It reduces the endogenous marrow content and should provide a greater chance for the incoming transduced, genetically corrected CD34⁺ cells to compete with the genetically defective bone marrow. This regimen of cytoxan is chosen because it has fewer side effects than total body irradiation (TBI) or the typical myeloablative regimen of higher dose cytoxan in combination with busulfan. In more than 20 patients on approved protocols, studied by Dr. Ball at the University of Pittsburgh, there have been no mortalities using a cytoxan dose of 7g/m². Other centers have successfully employed this regimen of cytoxan without mortality⁽⁹²⁾. In 146 cases reported in the literature, there has been one reported mortality. That seriously ill cancer patient died of infection during the period of cytopenia. Morbidity related to cytoxan include cytopenia, fever, bleeding, stomatitis and gastritis.

If cytoxan preparation is necessary, it will be done in patients after the results on measurements of transduction efficiency and testing for contamination of the infected CD34⁺ cells are complete and the cells released for use.

Patients undergoing this regimen will be studied for engraftment using the same criteria of enzyme activity in PBL and MØ markers as described in detail in this section.

M. RESPONSE CRITERIA

The experiment will be judged to be a success by the criteria listed below.

Group A Patients: Non-Enzyme Treated

- 1) An increase in hemoglobin of 2gm/dl
- 2) An increase in platelet count of 50%

- 3) A decrease in spleen size of 25%
- 4) A decrease in liver size of 25%
- 5) An improvement in MRI or X-ray of bone
- 6) A 50% decrease in serum non-tartrate inhibitable acid phosphatase
- 7) A 50% decrease serum angiotensin converting enzyme
- 8) An improvement in bone marrow morphology

Group B Patient: Enzyme Treated

Maintenance of improved clinical status without deterioration of one (1) year following the cessation of enzyme therapy.

N. ASSESSMENT OF RISK/BENEFIT

Gaucher disease is a progressive disorder. Most patients coming to medical attention suffer disability and a shortened life span. Allogeneic bone marrow transplantation is an option for only a few patients who have a matched sibling donor. Moreover, the risks of classical BMT are unacceptably high for many patients. Enzyme therapy ameliorates the disorder. However, it is not a permanent treatment, requires a life-long commitment to infusions of enzyme, and is very expensive. Gene therapy is a potential cure for the disease. If these initial studies are successful, they could provide treatment for essentially every patient with the disease. Without the need for myeloablation, the risks of gene therapy would be, in comparison to the potential benefit, very small. If partial myeloablation is required, the risk will be higher, but should be acceptable for patients with evident clinical disease. If complete myeloablation is necessary to achieve a satisfactory response, gene therapy would be reasonable only for the severely affected patient. The study proposed in this application will evaluate these possibilities.

The risk of malignancy to which the patient is put from the presence of replication competent retrovirus has now been estimated by a study in monkeys⁽⁹³⁾. It is clear that this risk is unacceptable. The risk of malignancy from replication defective retroviral vectors has not been estimated completely and will require many years of study to assess. To date, no malignancies have been reported in animal or human experiments employing replication defection retroviral vectors. With appropriate caution to avoid the introduction of RCR into patients or their cells, this risk should be minimized. In this application, we will take every precaution to detect the presence of RCR.

If the proposed studies are successful, they will have a significant impact on the treatment of Gaucher disease as well as a variety of other less common lysosomal storage diseases. Some of these can be treated with gene transfer to bone marrow stem cells. In others, development of vectors to target genes to other pathobiologically important cells will be required. If the proposed studies fail, they could provide guidance on how the approach might be improved.

The risks of the procedures are discussed below.

N.1 Contamination

Preparation of PBSC from blood will be performed in Dr. Ball's laboratory as is currently being done in a sterile fashion for studies of PBSC transplantation in cancer patients in IRB approved protocols. Transduction of PBSC will take place in a newly constructed Human Gene Therapy Application Laboratory in the Center for Biotechnology and Bioengineering. This laboratory will receive FDA approval for production and use of vectors to transduce cells for human gene therapy studies and treatment. This is a special area with stringent requirements for control of sterility. The cells used to generate vectors and the vector containing supernatants will be certified to be microbiologically clean and safe, and free of helper viruses.

The R-GC vector is a retroviral vector. It has the same risks associated with it as other retroviral vectors currently in use in clinical trials of gene therapy. In theory,

the risks of insertional mutagenesis or recombination to yield replication competent wild-type virus is low. Limited studies in non-human primates and man have suggested that these estimated are correct unless helper virus is present. The amphotropic MFG-GC vector has been tested and is helper virus free. Before the initiation of clinical trials, both the viral producer line and viral supernatants of the R-GC vector will be certified and approved by the FDA for use in human trials. Our own studies in many mice continuing for over a year, and a limited number of studies in dogs, indicate that the MFG-GC vector is safe.

N.2 Leukopheresis and G-CSF Priming

N.3 Partial Myeloablation With Cyclophosphamide

There are risks associated with the use of cyclophosphamide (Cytosan) to partially ablate the bone marrow. These include cytopenia, bleeding, infection, stomatitis, gastritis and alopecia (see earlier discussion).

N.4 Risks Associated With G-CSF Priming and Leukopheresis

No major adverse effects have been reported in published studies employing successive leukopheresis procedures. Serial leukopheresis may cause cytopenia, which may require postponement of the procedure for one or more days. Minor decreases in the hemoglobin may also occur. It is possible that patients with pre-existing anemia or thrombocytopenia may require the administration of red blood cells or platelets to restore adequate levels. Patients undergoing leukopheresis may experience symptoms of citrate toxicity, including parathesis, chills, abdominal discomfort, and nausea/ vomiting. Symptoms are routinely managed by making technical adjustments on the cell separator, and/or using calcium replacement (Ca gluconate) during the procedure. Severe reactions, which are rare, include tetany and seizure activity. Transient hypotension may also occur during the procedure.

Side effects associated with G-CSF therapy include bone pain (believed to be due to marrow expansion) and transient elevation of uric acid, lactate dehydrogenase, and alkaline phosphatase. Occasional mild hypotension has been observed. There have been no reports of allergic reactions, anaphylaxis, or antibody formation. The frequency of other adverse reactions, such as fever, nausea/vomiting, skin rash, dyspnea, and diarrhea, was not significantly different from placebo administration in clinical trials.

Possible risks associated with the use of central intravenous catheters, if used, include pneumothorax, bleeding, hypotension, air embolism, and bacterial infection, including sepsis.

The potential complications of reinfusion of cryopreserved PSC are comparable to those of cryopreserved bone marrow. These risks include nausea, vomiting, dimethyl sulfoxide (DMSO) odor, hypertension, hypotension, cardiac arrhythmia (brady- or tachycardia), volume overload, allergic reactions, and nephrotoxicity.

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Approved by Biomedical IRB,
University of Pittsburgh on

Consent to Act as a Subject in an Experimental Study
Competent Adults (18 years of age or older)

TITLE: Clinical Trial of Gene Therapy for Gaucher Disease

INVESTIGATORS: John A. Barranger, M.D., Ph.D.
Professor, Department of Human Genetics
Graduate School of Public Health
Dept. of Molecular Genetics & Biochemistry
School of Medicine
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Principal Investigator

Edward Ball, M.D.
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Co-Investigator

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Associate Professor
Dept. of Radiation Oncology
School of Medicine
University of Pittsburgh
Co-Investigator

SOURCE OF SUPPORT: National Institutes of Health; University of Pittsburgh

DESCRIPTION:

This study is being done to evaluate gene therapy as a treatment for Gaucher disease. The specific purpose is to see if we can restore the ability of cells in your bone marrow and blood to break down a lipid or "fat" that accumulates in them. Some years ago we showed that cells grown from a patient's skin could be made normal by putting a good gene for an enzyme into them. The good gene makes the enzyme (called glucocerebrosidase or GC for short) that your cells lack. The good gene overcomes the deficiency of GC that causes Gaucher disease. We have also shown that only a small amount of enzyme activity in only a few cells is enough to treat Gaucher disease. My laboratory showed this by making GC which was taken up by cells called macrophages. This is the name we use for the cells that eat up (phages) big (macro) molecules. The macrophages

John A. Barranger, M.D., Ph.D.

June 2, 1993

are very important in Gaucher disease. All of your cells lack sufficient amounts of glucocerebrosidase, but for some unknown reason, only macrophages store up the body lipid (a kind of a fat) that GC has the job of breaking down. So you see, macrophages play a central role in Gaucher disease and are responsible for the big liver or spleen and bone problems that you have. It is important that you know that these macrophages come from your bone marrow. The bone marrow is like a factory sending out cells to do various jobs in the body. One of those jobs is to clean up old cells and debris left behind by building up body parts or fighting the wars with bacteria and other foreign organisms. Again, these "clean up cells" or macrophages are sent out by your bone marrow all the time. Each macrophage does its job for about 3 months and then is itself replaced by a new one which comes from the bone marrow.

In Gaucher disease, the macrophages coming from the bone marrow lack a sufficient amount of this enzyme - GC - to do the job of breaking down this fatty material. We have shown that a new bone marrow from a donor can treat the disease. Unfortunately, many patients do not have a donor and the transplantation of donor marrow carries a significant risk. Estimates are that 10-25% of those patients receiving a donor transplant will die. Only in a few patients is this risk justifiable. Furthermore, enzyme replacement, while useful as a treatment, is not a cure and is expensive.

Our plan is to correct the deficiency of GC by adding a good gene to your bone marrow which contains the cells that reproduce and make macrophages for life. As I indicated, we have already corrected skin cells from Gaucher patients. We have also shown that we can get mouse bone marrow cells to make GC for a long time both in laboratory cultures and in whole mice. We have also shown that the good gene works in human macrophages to make GC the same way as it does in Gaucher patient skin cells and mouse bone marrow.

Revisions per your request

The first step in this study is to put a good gene for GC into cells that can make an entirely new bone marrow. We have learned that we can get these cells from your blood. They can be collected by connecting you to a machine which separates white blood cells (leukopheresis). The bone marrow-making cells are purified from the white blood cells in the laboratory. We will use methods in the laboratory to put the good gene into these cells. These methods involve the use of a mouse virus as a vehicle for carrying the gene into the cells. The virus has been altered so that to the best of our knowledge, it will not cause disease. However, there is a possibility that this virus could cause cancer or some other problem not yet identified. The risk of this occurring is difficult to estimate, but theoretically is a small one. So far, in animal and human studies, no problems have occurred, but you should know that not very many studies have been done and, thus, the experience is short.

Following these procedures to put the good gene into your bone marrow-making cells, we will put these genetically corrected cells into your blood stream. They will find their way into your bone marrow where they will make blood cells including the important macrophage. We do not know how many of your genetically corrected cells will be required to establish a population of cells that will result in a good clinical change in your condition. We reason that the

cells about 2 fold. We plan to use a dose of bone marrow-making cells that is usually used to replace the whole bone marrow. This dose may not work because your own bone marrow will be in competition with the new marrow. We will explain in the next paragraph what we will do if this happens. If the transplant does result in improvement in your enzyme system by 2 fold, we will follow you for about two years to see if the new marrow is making a difference in your disease.

If we can not increase the amount of enzyme in a patient's white blood cells, it is because their own bone marrow makes too many enzyme-deficient cells which overwhelm the good cells. In this event, we would use drugs to destroy the bone marrow and allow the new marrow to take over completely. If this is necessary, the risks are greater for the patient. Life-threatening complications can occur. These are discussed in more detail below. We will make the decision on whether or not it is necessary to use these drugs to destroy the bone marrow on the basis of the studies in the first two patients involved in this project. We will advise you of these results and our plans. If you are one of these first two patients, we will discuss the options again with you before proceeding further with the study.

Tests that will be done to assess your progress include blood tests, bone X-rays, special scans called magnetic resonance imaging (MRI) to measure the size of your organs and evaluate your bone problems, and a bone marrow biopsy. The blood tests will be done every month. The X-rays, scans, and bone marrow biopsy will be repeated every six months.

RISKS AND BENEFITS:

1. There is a risk that the gene placed in your cells will interfere with an important gene or activate an unwanted gene. This latter event could cause cancer or other presently unforeseen problems. While theory and a few studies indicate that this risk will be small, experience to date is limited.
3. The collection of the white cells from your blood (leukopheresis) requires that you have a large I.V. tube placed in a vein that goes into your chest. This is necessary to handle the large volume of blood from which the white blood cells are separated. There is a small chance of bleeding or accidentally entering the chest cavity. If this happens, a tube would need to be placed in your chest for several days. Careful placement of the catheter by an experienced person minimizes these risks which are quickly reversible should they occur.
4. In preparation for collecting the white cells discussed above, you would be treated with a special drug which stimulates the bone marrow to release bone marrow-making cells into the blood. This drug is called G-CSF. It can cause temporary bone pain during its use over a 10-20 day period. No major side effects of G-CSF have been reported.
2. Leukopheresis or collecting white blood cells requires connecting you to a machine which separates and collects white cells while at the same time returns red blood cells to you. There are no major side effects to this procedure. A potential complication is that

your red and white blood cell counts could be slightly low at the end of the procedure. However, close monitoring of your blood counts each morning will minimize this occurrence. The leukopheresis procedure will be repeated on five successive days.

5. If it is necessary to destroy your bone marrow to permit the genetically corrected marrow to be established, you will be treated with a drug called cyclophosphamide (also called cytoxan). There is about a 1% chance that the new marrow will not be established. To guard against this possibility, we preserve a sample of your marrow in the event it is needed. There are also risks associated with the use of cytoxan. These include sore mouth, irritation and bleeding of the urinary bladder, hair loss and irritation of the heart muscle which could lead to heart failure. Serious complications including life-threatening bleeding and infection can occur during the 4-6 weeks when the new bone marrow is being reestablished. The overall risk of mortality of autologous bone marrow transplantation to cancer patients is 10%. The risk in patients with Gaucher disease may be less.

ALTERNATIVE THERAPY

You may already be receiving enzyme therapy. You know that the treatment works to reduce the size of your organs and increase your blood counts. There is little risk to enzyme treatment. Gene therapy, if successful, could provide a lifelong cure for the disease. If you are receiving enzyme treatment, you should understand that our plan is to slowly withdraw enzyme treatment over a period of nine months. A major criterion of the value of gene therapy is whether or not withdrawal of enzyme can be tolerated. Furthermore, there is no certainty that the gene therapy approach being used in this study will be successful.

NEW INFORMATION

If new information, either good or bad, about this treatment comes to the attention of the investigator during the course of this study which may relate to my willingness to participate, it will be provided to me or my representative.

COSTS AND PAYMENTS

I will not be charged nor will I be paid for my participation in this study, although I will be charged for the (medical and hospital care) I require independent of this study just as though I were not part of this study.

COMPENSATION FOR ILLNESS OR INJURY

I understand that I will not be compensated for any injury or illness resulting from this study, but any emergency medical treatment which may be necessary will be provided.

CONFIDENTIALITY

I understand that any information about me will be kept in locked files with limited access and not distributed without my permission.

I understand that any information about me or my treatment will be treated in the same confidential manner as other hospital medical records. I consent to publication of any information for scientific purposes so long as my identity will not be revealed.

RIGHT TO WITHDRAW

I understand that I may refuse to participate in this study or withdraw any time and that my decision will not adversely affect my care at this hospital/institution or cause a loss of benefits to which I might otherwise be entitled. I also understand that I may be withdrawn from the study by the investigators.

VOLUNTARY CONSENT

Dr. Barranger has explained all of this to me and has answered all questions I have. I also understand that any future questions I have about this research will be answered by Dr. Barranger who I may call at 412/624-4623. Any questions I have about my rights as a research subject will be answered by the Office of the Senior Vice President for Health Sciences at the University of Pittsburgh. By signing this form, I agree to participate in this study.

Patient's Signature*

Witness

Date and Time

* The patient is unable to consent because:

I, therefore, consent to participation for the patient.

Relationship to Patient

Signature of Patient's Representative

Witness

Date

INVESTIGATOR'S CERTIFICATION

I declare that I have personally explained the above information to the patient or the patient's legal representative.

Investigator's Signature

Witness

Date

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

CVs

NAME BARRANGER, JOHN A.	POSITION TITLE Professor of Human Genetics
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EDUCATION (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
Loyola College, Baltimore, MD	B.S.	1967	Biology
University of Southern California Graduate School	Ph.D.	1973	Pharmacology
University of Southern California School of Medicine	M.D.	1974	Medicine

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

1974-1976 Intern and Resident, University of Minnesota, Department of Pediatrics
 1976-1978 Clinical Associate Development and Metabolic Neurology, RB, NINCDS, NIH
 1978-1986 Head, Clin. Investigations and Therapeutics Section, DMNB, NINCDS, NIH
 1983-1986 Acting Head, Molecular and Medical Genetics Section, DMNB, NINCDS, NIH
 1983-1986 Associate Chief, Development and Metabolic Neurology Branch, NINCDS, NIH
 1986-1990 Professor Pediatrics and Biochemistry, Univ. of Southern Cal., School of Medicine
 1990-Present Professor Human Genetics; Molecular Genetics and Biochemistry; Pediatrics, University of Pittsburgh

Honors: 1972 Phillip Aull Scholarship, 1973 Alpha Omega Alpha, 1973 March of Dimes Health Career Award, 1980 USPHS Commendation Medal, 1984 Arthur S. Flemming Award; Society for Pediatric Research, 1987.

Member: Biochemistry 2 Study Section 1986 - 1990; Chairman, Special Study Section: RFA on Gene Therapy, 1987; 1990, Co-Director, University of Pittsburgh, Gene Therapy Initiative; 1991, CHRC, Core Laboratory Director; 1992, Human Gene Therapy Applications Laboratory, Biotechnology Center, Director.

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Date of RAC Approval: 6/7/1993

Date of NIH Approval: 9/3/1993

Gaucher disease is an autosomal recessive storage disorder which causes an excess accumulation of glucocerebroside in bone marrow-derived cells called macrophages due to the functional absence of the enzyme glucocerebrosidase. Patients with Gaucher disease will be treated by gene therapy by inserting the cDNA for the normal glucocerebrosidase (GC) enzyme into hematopoietic stem cells (CD34+). Either G-CSF mobilized peripheral blood or bone marrow will be harvested from the patient and enriched for CD34+ cells. The CD34+ cells will be transduced with retroviral vector supernatant containing the cDNA for GC, over 72 hours in the presence of IL-3, IL-6 and stem cell factor. The transduced cells will be reinfused intravenously into the patient. Patients will be retreated four times 2 - 4 months apart if peripheral blood stem cells are treated. Bone marrow will be treated only one time. The patient will be monitored for any evidence of toxicity and for the number of cells containing the retroviral vector. If enough cells are found to contain the retroviral vector the cells of the subjects will also be evaluated for the expression of GC. It is hoped that the information obtained from this study will permit the development of gene therapy for Gaucher disease.

Gaucher disease causes fat to collect in bone marrow-derived cells called macrophages. Fat collects there because of an abnormality in an enzyme called glucocerebrosidase. Gaucher disease results in many health problems. Current treatment for this disease involves repeated intravenous treatment with normal enzyme. This treatment is called enzyme replacement therapy. Another treatment for Gaucher disease is bone marrow transplantation. Bone marrow transplantation involves destroying a person's bone marrow with drugs and irradiation and replacing it with someone else's bone marrow. This treatment has many risks but can cure the patient for the rest of their life. It would be good to be able to treat people with Gaucher disease with a treatment like bone marrow transplant but without the risks connected with destroying bone marrow cells and using someone else's bone marrow. Either some blood or bone marrow will be obtained. A special type of cell (stem cell) that makes all the other cells in both the blood and bone marrow including macrophages will be isolated in the laboratory. These cells will be isolated in the laboratory and used for "gene therapy". New genetic material will be put into these special stem cell using a specially modified virus called a murine retrovirus vector. The murine retrovirus has been altered so it can deliver new genetic material to a cell but not cause an infection. The new genetic material inserted into your cells using the retrovirus vector will serve as a blueprint to allow the cells to make the enzyme that was abnormal. After putting the new genetic material into the cells, these cells will be put back into the patient by needle injection into a blood vessel. This will be done without first destroying the patient's bone marrow cells. If bone marrow cells are used to obtain the stem cells, only one treatment will be done. If blood is treated, four treatments every 2 - 4 months will be done. The patients will be watched for any unexpected harmful side effects that may result from this therapy. Samples of blood and marrow cells will be taken periodically to measure the number of cells that contain the new genetic material. If enough cells contain the new genetic material, the amount of enzyme being produced will be measured. It is hoped that this study will provide doctors with more information which might permit them to develop a safe and effective gene therapy for persons with Gaucher disease.

**Retroviral Mediated Transfer of the cDNA for Human
Glucocerebrosidase into Hematopoietic Stem Cells of Patients
with Gaucher Disease**

Protocol GTI 0101

Sponsored by:

**Genetic Therapy, Inc
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PROTOCOL ROSTER

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PROTOCOL SYNOPSIS

- Title:** Retroviral Mediated Transfer of the cDNA for Human Glucocerebrosidase into Hematopoietic Stem Cells of Patients with Gaucher Disease
- Sponsor:** Genetic Therapy, Inc.
- Study Design:** This is a Phase I Study to investigate the feasibility and safety of transferring glucocerebrosidase (GC) cDNA into hematopoietic stem cells of patients with Gaucher disease which is a genetic disorder characterized by the accumulation of glucocerebroside.
- Patient Population:** Patients $\geq$ 18 years of age with Gaucher disease.
- Sample Size:** 10 evaluable patients
- Treatment:**
- A. First treatment cycle consists of bone marrow (BM) harvest OR mobilized peripheral blood stem cell (PBSC) harvest.
 1. Conventional marrow harvest is used; OR
 2. PBSC harvest:
 - a. G-CSF: 10 μ g/kg/day (1X) SQ for 7 days
 - b. PBSC harvested days 5, 6, and 7 from patient and processed.
 - B. BM or PB cells enriched for CD34+ cells enriched with immunoabsorption column (CellPro Inc.)
 - C. Cells transduced with Gc vector-containing supernatant.
 - D. Cells reinfused into patient.
 - E. $\leq$ 4 cycles of PBSC transduced cells, no more frequent than every 2 months. No further cycles if first cycle was bone marrow harvest.
- Study Endpoints:**
1. Development of high grade toxicities or intolerances.
 2. Expression of GC enzyme levels equivalent to normal patients
- Study Objectives:**
- To investigate whether the cDNA encoding human glucocerebrosidase can be safely transferred to hematopoietic stem cells by retroviral mediated gene transfer.
 - To determine the extent of engraftment after autologous transplantation of transduced hematopoietic stem cells into individuals with Gaucher disease without prior ablation (partial or complete) of endogenous marrow.
 - To investigate whether the transferred human glucocerebrosidase cDNA will be expressed in monocytes/macrophages at levels that might be expected to be beneficial.
 - To investigate the effect of repeated transplantation of transduced PBSC into Gaucher disease patients on the fraction of monocyte/macrophages containing and expressing glucocerebrosidase.
- Protocol Co-chairs:** Donald Kohn, MD, Stefan Karlsson, MD

SCHEMATIC

Bone Marrow Harvest - 1 Cycle only

Patients with Gaucher Disease

|

Harvest Bone Marrow Cells
Filter and process to recover CD34-enriched Cells
(Day 1)

|

Discharge patient from hospital if clinically indicated
(Day 2)

|

Transduce CD34+ cells with GC-gene containing retroviral vector
(Days 1-3)

|

Transduced cells reinfused into patient
(Day 4)

|

Patient Discharged
(Day 5)

|

Patient Follow-up

SCHEMATIC

Peripheral Blood Stem Cell Harvest

Patients with Gaucher Disease

|
G-CSF 10 ug/kg/day
(Day 1 - 7)

|
Peripheral Blood Stem Cell Harvest
Filtered and processed to recover CD34-enriched Cells
(Days 5, 6, and 7)

|
Transduced with GC-gene containing retroviral vector(s)
(Days 5 - 10)

|
Transduced cells reinfused into patient
(Day 8 - 10)

|
Patient Discharged
(Day 11)

|
Patient Follow-up
(end of Cycle 1)

|
Repeat up to 3 more cycles

ABSTRACT

Patients with Gaucher disease suffer from a lack of functional glucocerebrosidase (GC). Disease symptoms are a result of macrophage engorgement secondary to this enzyme deficiency. This study is designed to determine if the cDNA encoding normal GC can be introduced into macrophage precursors using a retroviral vector. CD34+ cells obtained from G-CSF mobilized peripheral blood stem cells or from bone marrow will be transduced *ex vivo* over a 72-hour period in the presence of IL-3, IL-6, and Stem Cell Factor with a GC retroviral vector. These transduced cells will be reinfused into the patient and the patient monitored for toxicities as well as evidence of successful gene transfer and expression. If peripheral blood stem cells are used this procedure will be repeated a total of 4 times with each treatment 2 - 4 months apart. If bone marrow is used, only one treatment with gene-modified cells will be used.

Ten patients will be enrolled in the protocol. Two sites will be involved, the National Institutes of Health and Childrens Hospital of Los Angeles.

1.0 BACKGROUND AND RATIONALE

1.1 Background: Gaucher Disease

Gaucher disease is an autosomal recessive storage disorder characterized by the accumulation of glucocerebroside. Dr. Philippe Gaucher originally described a patient in 1882 whose spleen and liver were greatly enlarged. It is now understood that these organs are enlarged in Gaucher disease secondary to accumulation of glucocerebroside in the lysosomes of macrophages (1, 2). In 1965, Dr. Roscoe Brady and his co-workers demonstrated that the accumulation of glucocerebroside occurred because an enzyme, glucocerebrosidase, was deficient in these individuals (3, 4). The storage or accumulation of glucocerebroside in the lysosomes of macrophages results in massive macrophage engorgement which is responsible for most of the morbidity and mortality associated with Gaucher disease. This process is most marked in spleen, liver and bone marrow. Spleen involvement often results in a massively enlarged spleen with accompanying anemia, thrombocytopenia, and neutropenia. These same three problems are exacerbated by the bone marrow involvement in Gaucher disease. Marrow involvement can also lead to bone pain, skeletal malformations, and pathologic fractures. Liver involvement can lead to massive enlargement and occasionally cirrhosis.

Other organs besides liver, bone marrow and spleen can be affected in Gaucher disease. The enlarged spleen and liver may make breathing more difficult. The lungs themselves may be directly involved resulting in decreased gas exchange. The heart can be overworked pumping large quantities of blood to enlarged organs. The skin can show changes related to glucocerebroside storage. The most important other organ involvement is the central nervous system. Gaucher disease is divided into three types based on the extent of nervous system involvement. The most common type of Gaucher disease (Type I) does not have neural involvement and therefore is sometimes referred to as non-neuronopathic Gaucher disease. Type 2 Gaucher disease has severe neurological symptoms and patients usually do not live past the age of two. Type 2 (infantile Gaucher disease) fortunately is very rare. Type 3 Gaucher disease has slowly progressive neurological symptoms and these patients may live to the age of forty years. Although more common than Type 2, Type 3 is rare with a cluster in Sweden.

1.2 Background: Treatment for Gaucher Disease

Treatment of Gaucher Disease With Bone Marrow Transplantation

For many years there was only supportive treatment available for people with Gaucher disease. Research has emphasized developing treatment focussed on the macrophages and their storage of glucocerebroside. Since the cell that is responsible for the pathology seen with the disease is the macrophage, attempts have been made to replace the defective macrophages. Since macrophages are bone marrow-derived, bone marrow transplantation has been used to replace the defective macrophages with new marrow-derived macrophages containing functional glucocerebrosidase enzyme. The results on patients receiving and surviving the marrow transplant procedure are encouraging. The symptoms secondary to spleen and bone marrow involvement have been alleviated (5-9).

Progression of bone symptoms has been greatly alleviated except for pre-existing permanent deformities (5-7). It is particularly encouraging that the CNS symptoms of a Type 3 patient from Sweden have dramatically improved after marrow transplantation and the patient now has normal intellectual function (Professor Ollé Ringdén, personal communication, see also references 6 and 7).

Marrow transplantation is performed most effectively if a normal HLA-identical sibling is used as a marrow donor although significant transplant-related morbidity and mortality still occur in a minority of these patients. A majority of Gaucher patients will not have an HLA-identical sibling to serve as donor. Non-HLA-matched donors and HLA-matched unrelated donors could be used, but the morbidity and mortality risk is much greater than that seen with patients having an HLA-matched sibling donor. Because of the cost, both in terms of health risks as well as monetary terms, marrow transplantation has not been widely utilized in patients with Gaucher disease.

Enzyme Replacement Therapy for Gaucher Disease

Since a curative bone marrow transplant is associated with significant risks, alternative therapies have been tested. Dr. Brady and his co-workers have developed a placenta-derived glucocerebrosidase enzyme which has been modified so that it "targets" to macrophages (10-12). This modified enzyme is given to patients to replace the defective or absent glucocerebrosidase in their macrophages. The repetitive administration of the modified enzyme has been associated with clinical reversal of many of the disease's effects. Enzyme replacement therapy is effective and safe, but enzyme replacement therapy is very expensive with the enzyme costing \$3.5/I.U. and the usual starting dose for a Type 1 patient being 60 IU/kg given intravenously every two weeks. For a 70-kg adult the expense for enzyme alone would be almost \$400,000 per year. The dose may be decreased in future trials, perhaps starting off at one-fourth the dose; this would reduce expenses by the same amount but the therapy would still be very expensive. It is hoped that once the majority of glucocerebroside in the patient is cleared, a lower maintenance dose could be given. Whether an effective maintenance dose would reduce yearly expenses much below \$100,000 per year is not known. The enzyme replacement is an effective therapy for Type 1 Gaucher disease patients but effects on the CNS symptoms in the rarer Type 3 patients are not yet known.

Gene Therapy for Gaucher Disease

An alternative form of curative therapy could be gene therapy. Retroviral mediated gene transfer (RMGT) has been used to transfer glucocerebrosidase cDNA into Gaucher-patient derived cells. The enzyme deficiency in hematopoietic cells from Gaucher patients has been corrected following retroviral mediated gene transfer into CFU-GM progenitor cells (13). High efficiency transfer into more primitive hematopoietic progenitor cells has also been seen (14). Both studies reported enzyme levels in the patient cells that are equivalent to those found in normal individuals. By transferring the glucocerebrosidase cDNA into patient marrow progenitor cells *ex vivo* and then returning the primitive cells to the patient it may be possible to let the patient serve as their own bone marrow stem cell donor. Autologous marrow transplantation is associated with

lower risks than allogeneic transplantation. The risks associated with autologous transplantation are from the ablative conditioning therapy. If individuals with Gaucher disease could be transplanted with primitive macrophage precursor cells and stem cells into which a functional glucocerebrosidase gene has been placed without requiring a dangerous ablative regimen, the risk would be greatly reduced. It is the goal of gene therapy to be able to modify the patients own macrophage precursor cells and return them to the patient without using dangerous ablative regimens. If enough of the patient's macrophages could be corrected, gene therapy would be therapeutic. If enough multi-potential pluripotent hematopoietic stem cells were corrected, gene therapy would be expected to be curative, as is allogeneic bone marrow transplantation.

1.3 Preclinical Data

Retroviral mediated transfer of GC cDNA in rodent *in vivo* marrow transplant models

Extensive studies by several investigators have demonstrated the feasibility of retroviral mediated gene transfer into the hematopoietic stem cells of mice (15, 16). Furthermore, efficient transfer of GC cDNA into a substantial fraction of murine hematopoietic stem cells has been accomplished using retroviral mediated gene transfer (17-20). The retroviral constructs utilized are similar or identical to the ones proposed to be used clinically in this protocol. The Gc vector is equivalent to the LG vector described by Dr. Karlsson (20, 25) and the L-GC vector described by Dr. Kohn (14). Efficient transfer requires the presence of growth factors during the transduction period; in particular, Stem Cell Factor (SCF), interleukin-3 (IL-3) and interleukin-6 (IL-6) have been shown to increase gene transfer efficiencies. The sustained long-term level of human glucocerebrosidase expression measured in these animals is substantially higher than endogenous murine enzyme levels and would therefore be expected to permit correction of the enzyme defect in Gaucher disease patients if similar levels were obtained in humans.

Retroviral mediated transfer of GC cDNA into human hematopoietic cells

RMGT into human colony-forming cells has advanced significantly over the efficiencies seen only a few years ago. Using combinations of growth factors such as interleukin-1 (IL-1), IL-3, IL-6, and SCF it has been possible to increase the efficiency of gene transfer into progenitor cells to approximately 50% or more (21-25). These efficiencies have been seen with a variety of retroviral vectors transferring a variety of genes. Moreover, the efficient transfer of GC to cells derived from patients with Gaucher disease has been directly demonstrated and the expression of adequate levels of gene product shown (13, 14). This data includes the transfer and expression of GC in Gaucher disease macrophage precursor cells.

Retroviral mediated transfer of GC cDNA into human long-term marrow cultures

Human colony-forming assessments of gene transfer are limited in their ability to measure the parameters of gene transfer into more primitive hematopoietic cells. To study earlier progenitor cells (and possibly the pluripotent stem cell), Dexter developed

a long-term culture system to study early murine hematopoietic cells. A number of researchers have successfully modified the conditions to permit similar studies on human marrow. The results of using such a model system have been encouraging as results similar to that seen in colony-forming cells have been seen in long term marrow culture initiating cells (14). The GC gene itself can be transferred and expressed in appropriate cells as determined in this model system.

Large animal experience with retroviral mediated gene transfer into marrow cells

A number of studies have been published on gene transfer into bone marrow cells of outbred animals followed by autologous transplantation (reviewed in reference 16). Bone marrow stem cells of rhesus monkeys have been successfully transduced by two groups (26-28). Multilineage reconstitution of transduced cells was reported and long-term expression of the transferred gene obtained (27). Rhesus CD34+ cells have been transduced and autologous transplantation with these cells has led to long-term reconstitution with Neo^R-gene marked cells (28). Similar results have been obtained transducing rhesus CD34+ cells with an ADA gene vector (Bodine D, Williams D, Donahue R, and Nienhuis A, personal communication). Hematopoietic stem cells from dogs (29) and cats (30) have also been transduced successfully. The GC gene has been transferred into rhesus hematopoietic cells and gene transfer into CD34+ progenitor cells has been very efficient *in vitro* (2 animals) using a supernatant infection with IL-3, IL-6 and stem cell factor. Autologous marrow transplantation of rhesus CD34+ cells which were transduced with peripheral blood and bone marrow have been followed for up to four months (unpublished studies).

1.4 Clinical Data

Human experience with retroviral mediated gene transfer into marrow cells

In order to study the role of autologous marrow in relapses of patients undergoing autologous bone marrow transplantation (ABMT) for either acute myelogenous leukemia or neuroblastoma, harvested marrow has been marked by RMGT. These ongoing studies have not only been fruitful in determining the role of infused autologous marrow in contributing to the relapse (31), but also in demonstrating RMGT into hematopoietic cells. Patients have now been followed for up to one year after ABMT. Analysis of these patients has shown gene transfer into all lineages of marrow-derived cells including monocytes, neutrophils, and T and B lymphocytes. Also encouraging is the detection of transcribed RNA from the retroviral vector transferred into these cells. The human studies, using vectors very similar to those to be used in this study but expressing a bacterial antibiotic resistance gene, imply that RMGT into very early hematopoietic progenitor cells is possible and that the expression is preserved long-term. Since the marrow was not cultured in the presence of growth factors during the transduction period, the gene transfer efficiency in these studies may be lower than would be expected using the procedures proposed in this protocol.

Ability to transfer marrow progenitor cells into non-ablated recipients

Using mouse models, investigators have been able to demonstrate the successful transfer of marrow stem cells into recipient mice without first "making space" with ablative treatments (32-36). These studies also have demonstrated that these non-ablative transfers can be repeated with a cumulative effect. Based on these rodent experiments it is rational to expect engraftment of autologous cells removed from a patient and treated *ex vivo* with retroviral vectors if the patient's marrow is not ablated.

Some human diseases have been successfully treated with similar non-ablative marrow transplantation regimens. These diseases include the allogeneic transplantation of marrow into patients with T-cell or severe combined immune deficiencies (ADA negative SCID, ADA positive SCID, Short-limbed dwarf). In these patients the donor cells have engrafted successfully and corrected the underlying immune defect. The donor marrow can be shown to have repopulated the immune system (T-cells) preferentially. Donor engraftment in other compartments is more difficult to demonstrate but has been seen. The ability to serially engraft patients with repetitive transplants has been shown in some of these diseases as well. In one form of immune deficiency, a patient with Wiskott-Aldrich syndrome who received a transplant from his father after ablation remained chimeric and thrombocytopenic. This patient then received marrow from his father again without ablation. This transplant was shown to engraft by HLA and antigen studies of various marrow populations as well as platelets (Horowitz, personal communication). The patient's thrombocytopenia was corrected with platelets of parental origin. These human experiences support the concept that "making space" is not absolutely necessary to obtain engraftment of marrow cells.

1.5 Rationale/Justification

As outlined in the previous sections, retroviral vectors can efficiently transfer and express the GC (glucocerebrosidase) cDNA into rodent, primate and human hematopoietic cells (including Gaucher disease patient cells). The level of expression is appropriate for therapeutic benefits and the gene is expressed in the appropriate target cells (monocyte/macrophages). Clinical experience with similar vectors also demonstrate the utility of RMGT (retroviral mediated gene transfer) to transduce and express in the appropriate target cells. Human and animal experience suggests that it should be possible to engraft hematopoietic stem cells without requiring ablation. All components necessary to execute the plan in this protocol have been demonstrated in both animal and human protocols.

It is estimated that there is one stem cell in every 10^5 murine mononuclear bone marrow cells. If this ratio of total bone marrow cells to stem cells applies to humans, then we will be transducing an average of $1-10 \times 10^4$ stem cells from each patient. Even with a transduction efficiency of 1% or lower, it would be likely that we would be able to detect stem cell transduction in each patient as if it was actually occurring *in vitro* under these transduction conditions.

It is not anticipated that this study will have a sufficient therapeutic benefit to the patient to permit the removal of enzyme replacement therapy immediately, but it is possible that the Gaucher disease patients involved in the study will express significant amounts of glucocerebrosidase to permit the consideration of discontinuing enzyme replacement therapy. The study is expected to assist in the development of gene therapy for Gaucher disease so it will be unnecessary to continue enzyme replacement therapy. This study represents a step towards reaching that goal.

1.51 Biology of reconstitution following autologous transplantation of bone marrow or peripheral blood stem cells

As is true in all genetic marking protocols or gene therapy protocols, very little can be learned if marking is completely unsuccessful, and no transduced cells can be identified either short or long term after transplantation and engraftment. The previously described experimental data imply that significant transduction will occur. What is not as fully tested in humans is the ability to engraft early hematopoietic progenitor and stem cells in individuals without prior ablative therapy. The ability to perform autologous marrow transplantation with gene altered marrow without ablative therapy would greatly enhance the use of gene transfer techniques to study and treat human disease. The proposed protocol is designed to provide information about the biology of reconstitution following autologous transplantation.

1.6 Objectives

- 1.61 To investigate whether the cDNA encoding human glucocerebrosidase can be safely transferred to hematopoietic stem cells by retroviral mediated gene transfer.
- 1.62 To determine the extent of engraftment after autologous transplantation of transduced hematopoietic stem cells into Gaucher disease patients without prior ablation (partial or complete) of recipient marrow.
- 1.63 To investigate the whether the transferred human glucocerebrosidase cDNA will be expressed in monocytes/macrophages at levels that might be expected to be of therapeutic benefit.
- 1.64 To investigate the effect of repeated transplantation of transduced peripheral blood stem cells into individuals with Gaucher disease upon the fraction of monocyte/macrophages containing and expressing glucocerebrosidase.

2.0 STUDY DESIGN

2.1 Overview

This will be a Phase I, two-center study. Participants will at least 18 years of age with a diagnosis of Gaucher disease. Two sources of hematopoietic stem cells will be used, bone marrow or growth-factor mobilized peripheral blood stem cells (PBSC). Marrow harvest will be followed by enrichment of the CD34+ subpopulation. For PBSC harvest, G-CSF will be given for 7 days and the patient observed closely for adverse reactions. PBSC will be collected on days 5, 6 and 7 and cells will be processed for enrichment of CD34+ cells. Each time cells (marrow or PBSC) are collected they will be cultured and subsequently transduced with the GC-cDNA containing retroviral vector(s). These transduced cells will be reinfused into the patient.

2.2 Number of Patients/Patient Management

Ten participants will be enrolled into this study.

Patients receiving PBSC infusions will need to be hospitalized approximately 10 days (or be seen daily for several hours) every two to four months for approximately one year. Patients will be monitored daily while being treated, and monthly between cycles and every 3 months during follow-up (as an out-patient). Patients will be reinfused up to a total of 4 times (4 cycles). As patients are not expected to develop significant therapeutic effects from the gene transfer, the monitoring will emphasize toxicities. Efficacy will be evaluated by monitoring for the presence of gene transfer into hematopoietic cells. Expression of the gene product will be monitored by RNA analyses to detect the presence of mRNA from the transferred cDNA sequence. Protein expression will be monitored by glucocerebrosidase enzyme activity. If the patients are on enzyme replacement therapy the routine monitoring for this treatment will overlap with additional tests related to the gene transfer aspects.

For patients receiving the single bone marrow infusion, hospitalization periods will be about three days for marrow harvest and one to two days for cell infusion. Cell infusion may alternatively be given on an outpatient basis. Patient follow-up will be the same as for patients receiving PBSC infusions.

2.3 Source of Patients/Participating sites

This study will be conducted at NIH and CHLA. Consecutive potentially eligible participants will be evaluated to reduce selection bias. Both men and women will be eligible to participate in this study.

2.4 Study Duration

The bone marrow harvest and infusion will occur in less than one week. Each PBSC cycle will take approximately two weeks to complete. PBSC patients will be asked to have these cycles repeated no more than every 2 months and there may be 4 cycles total.

Upon completion of study treatment (marrow or PBSC), patients will continue to be followed monthly for up to 1 year, every six months for the next year.

3.0 PATIENT SELECTION

3.1 Inclusion Criteria

Patients must meet the following criteria within 14 days prior to study entry (Day 0) unless otherwise noted:

3.11 Men or women, age ≥ 18 years of age with confirmed Gaucher disease

3.12 Established diagnosis of Type I Gaucher disease as confirmed by:

3.121 Glucocerebrosidase enzyme level determination, OR

3.122 DNA sequence analysis

3.13 Adequate baseline organ function as assessed by the following laboratory values within 30 days prior to study entry (day -30 to 0):

- o Adequate renal function with estimated creatinine clearance > 50 ml/min; which can be estimated by the following formula:

$$\text{CrCl male} = \frac{(140 - \text{age in years} \times (\text{body weight in kg}))}{(\text{SCr}) \times (72)}$$

$$\text{CrCl female} = 0.85 \times (\text{CrCl male})$$

- o Adequate liver function with SGOT, SGPT and alkaline phosphatase ≤ 5 times the ULN [if transaminases $>$ than the upper limit of normal (ULN), patients should have a hepatitis B surface antigen (HBsAG) test prior to study entry. Patients may not enter the study if HBsAG is positive.]
- o PT and PTT not more than 1.5 times the ULN
- o Hemoglobin ≥ 9.0 g/dl (patients cannot be transfusion dependent)
- o Platelets $\geq 75,000/\text{mm}^3$
- o WBC $> 2,000/\text{mm}^3$
- o Absolute Neutrophil Count (ANC) $\geq 1,000/\text{mm}^3$
- o Serum Amylase ≤ 1.5 times the ULN

- o Bilirubin $\leq$ 2.5 mg/dL
- o Triglyceride < 400 mg/dl
- o ESR < 1.5 times the ULN

3.14 Ability to give informed consent

3.15 Normal cardiac function by history and physical exam

3.16 Resting transcutaneous oxygen saturation > 90% on room air

3.17 Karnofsky Performance Status $\geq$ 40 (see Appendix IV)

3.18 If not treated with Ceredase, spleen size > 10X normal

3.2 Exclusion Criteria

Patients who meet any one of the following criteria will be excluded from study entry:

- 3.21 Pregnant or lactating females: all patients must practice adequate birth control and females of child-bearing potential must have a negative serum beta-HCG pregnancy test within Study Days -7 to 0.
- 3.22 Acute infection: any acute viral, bacterial, or fungal infection which requires specific therapy. Acute therapy must have been completed within 14 days prior to study treatment.
- 3.23 Hepatitis-B surface antigen positive patients
- 3.24 HIV-infected patients
- 3.25 Acute medical problems such as ischemic heart or lung disease that may be considered an unacceptable anesthetic or operative risk
- 3.26 Patients with any underlying conditions which would contraindicate therapy with study treatment (including allergies to reagents used in this study)
- 3.27 Patients that have had total splenectomy performed and have significant marrow involvement as determined by MRI are excluded from receiving G-CSF and undergoing peripheral stem cell harvesting.

- 3.3 Concomitant Treatment/Procedures: All allowed medications or treatments should be kept to a minimum and recorded. All questions regarding concomitant medications should be referred to the study chair or investigator.

Use of Ceredase: Patients taking Ceredase prior to study entry will be permitted to participate in this trial. Patients will be allowed to continue Ceredase therapy if deemed clinically necessary.

4.0 CLINICAL AND LABORATORY EVALUATIONS

As patients may not receive significant therapeutic effects from the gene transfer, the monitoring will emphasize safety (if toxicity is found refer to section 6.0 and Appendices II and III). Study patients may also be on enzyme replacement therapy so the routine monitoring for this treatment may overlap with additional tests related to the gene transfer aspects.

4.1 Screening and Baseline Evaluations

The following clinical and laboratory evaluations will be performed for all study participants within Study Days -30 to 0 unless otherwise noted. Screening evaluations, or evaluations that determine patient eligibility, are noted. These screening evaluations must be reviewed prior to initiation of study treatment.

NOTE: Day 0 and 1 may be the same date or up to 24 hours apart, however Day 1 begins with treatment initiation. Any *baseline* evaluations (*Day 0*) which are performed on the day treatment will begin (*Day 1 of treatment*) must be performed prior to treatment.

- 4.11 Objective Signs and Symptoms: include vital signs (blood pressure, pulse, temperature and respirations). (Screening and repeat Day 0)
- 4.12 History and Physical Examination: Complete history and comprehensive physical will be performed for screening. A targeted physical exam will be done Day 0.
- 4.13 Karnofsky Performance Status (see Appendix IV) (Screening)
- 4.14 Chest X-Ray: Normal CXR within the last 30 days unless new symptoms suggest that this be repeated prior to dosing (Screening)
- 4.15 Electrocardiogram (ECG): ECG must be normal within 30 days unless new symptoms suggest this be repeated prior to dosing (Screening)
- 4.16 Urinalysis: Routine screening urinalysis including creatinine and creatinine clearance (Screening and repeat Day 0)

- 4.17 Serum Chemistries: include uric acid, calcium, phosphorus, magnesium, amylase, transaminases (SGOT, SGPT), alkaline phosphatase, LDH, total bilirubin, triglycerides, BUN, creatinine, albumin, total protein, electrolytes, and glucose (Screening and repeat Day 0)
- 4.18 Hematology: Complete blood count (CBC), differential, platelets, ESR, and coagulation tests should include PT (Prothrombin Time), PTT (Partial Thromboplastin Time), and fibrinogen (Screening and repeat Day 0).
- 4.19 Pregnancy Test: Serum pregnancy test must be negative within 7 days prior to Day 0, or between study days -7 and 0.
- 4.110 HIV Negative Antibody Test: ELISA (Screening)
- 4.111 HBsAG: Hepatitis B surface antigen determination (Screening)
- 4.112 Liver-Spleen Size by MRI or CAT: Optional: To be done if clinically indicated.
- 4.113 Resting transcutaneous oxygen saturation: Must be > 90% on room air
- 4.114 Glucocerebrosidase enzyme level: collect PBMCs for this determination (Screening)
- 4.115 MoMLV Antibody Analysis: (Day 0)
- 4.116 Freezing Specimens: Additional serum, plasma, and blood cells should be separated and frozen at baseline (Day 0).
- 4.117 DNA/RNA Analysis: Circulating mononuclear cells will be analyzed using PCR-related techniques for the presence of vector gene sequences (Day 0, baseline).
- 4.118 Marrow MRI Analysis: Analysis of marrow involvement will performed using T1 weighted magnetic resonance imaging techniques if clinically warranted.

4.2 Evaluations During Treatment:

Evaluations are to be done *prior to* that days procedures or treatments.

Note for Marrow Harvest:

Day M1: Marrow harvest

Day M2: Patient discharged if clinically indicated

Days M1, M2, and M3: CD34+ cell transduction *ex vivo* with Gc

Day M4: Transduced marrow reinfusion (in- or out-patient)

Day M5: Patient discharge

Note for Peripheral Blood Stem Cell (PBSC) Harvest:

Days P1 - P7: Begin administration of G-CSF to patient

Days P5, P6, and P7: PBSC harvested

Days P5 - P10: PBSC transduction *ex vivo* with GC vector

Days P8, P9, and P10: Transduced cells reinfused

Day P11: Patient discharge

- 4.21 Objective signs and symptoms: Vital signs (blood pressure, pulse, temperature and respirations) will be done Study Days M1, M2, M4, M5 and P1 through P11. Weight will be done day of discharge (M5, P11)
- 4.22 Physical Examination: Target physical exam will be repeated day of discharge (M5, P11).
- 4.23 Karnofsky Performance Status (see Appendix IV) will be done day of discharge (M5, P11).
- 4.24 Urinalysis: Routine urinalysis, including creatinine and creatinine clearance, will be done Day M1, M2, M4, and P1-10 and day of discharge (M5, P11).
- 4.25 Serum Chemistries: include uric acid, calcium, phosphorus, magnesium, amylase transaminases (SGOT, SGPT), alkaline phosphatase, LDH, total bilirubin, triglycerides, BUN, creatinine, albumin, total protein, electrolytes, and glucose will be done Study Days M1, M2, M4, and P1-P10 and day of discharge (M5, P11).
- 4.26 Hematology: Complete blood count (CBC), differential, platelets, and coagulation tests should include PT (Prothrombin Time), PTT (Partial Thromboplastin Time), and fibrinogen will be done Study Days M1, M2, M4 and P1-P10 and day of discharge (M5, P11).
- 4.27 Pregnancy Test: Females will have a serum pregnancy test repeated monthly during the first year.
- 4.28 Liver-Spleen Size by MRI or CAT: Optional, if clinically indicated.
- 4.29 Glucocerebrosidase enzyme level determination: Obtain PBMC on day of discharge.
- 4.210 MoMLV Antibody Analysis: Sample obtained day of discharge (Day M5, P11).
- 4.211 Freezing Specimens: Additional serum, plasma, and cells should be separated and frozen day of discharge (Day M5, P11).
- 4.212 DNA/RNA Analysis: Circulating mononuclear cells will be analyzed using PCR-related techniques for the presence of vector gene sequences day of discharge.

4.3 Evaluations Between PBSC Treatment Cycles:

For patients completing each PBSC cycle, the following evaluations will be done 1 month after patient is discharged and repeated monthly until the next cycle begins. See section 4.4 if patient is completely done with all of the study therapy or has completed at least 1 cycle (and has subsequently discontinued):

- 4.31 Objective signs and symptoms: Vital signs (blood pressure, pulse, temperature and respirations) weight and height .
- 4.32 Physical Examination: Target physical exam
- 4.33 Karnofsky Performance Status (see Appendix IV).
- 4.34 Urinalysis: Routine screening urinalysis including creatinine and creatinine clearance.
- 4.35 Serum Chemistries: include uric acid, calcium, phosphorus, magnesium, amylase transaminases (SGOT, SGPT), alkaline phosphatase, LDH, total bilirubin, triglycerides, BUN, creatinine, albumin, total protein, electrolytes, and glucose.
- 4.36 Hematology: Complete blood count (CBC), differential, platelets, and coagulation tests should include PT (Prothrombin Time), PTT (Partial Thromboplastin Time), and fibrinogen.
- 4.37 Pregnancy Test: Serum pregnancy test will be repeated monthly during the first year.
- 4.38 Liver-Spleen Size: Optional: based on clinical assessment.
- 4.39 Glucocerebrosidase enzyme level determination: Collect PBMCs.
- 4.310 Antibody Analysis: Serum sample for anti-retroviral antibodies.
- 4.311 Freezing Specimens: Additional serum, plasma, and cells should be separated and frozen.
- 4.312 DNA/RNA Analysis: Circulating mononuclear cells will be analyzed using PCR-related techniques for the presence of vector gene sequences.

4.4 Post-Treatment Evaluations for Patients Completing Marrow Treatment or 1 or more PBSC Cycles

Follow-up evaluations will be done on all patients who have completed at least 1 treatment.

See section 4.3. Evaluations will be similar to patients who are between cycles, however patients will now be seen at least every month for one year, every three months for the next year and then yearly unless there is a clinical reason to see the patient more often. In addition to the evaluations outlined in section 4.3 the following will also be done:

4.41 DNA/RNA Analysis: Peripheral blood samples (20 cc green top) for DNA and RNA analysis.

4.42 GC Enzyme Analysis: Peripheral blood sample (20cc green top) for enzyme analysis.

4.43 Bone Marrow Aspirate: For DNA/RNA analysis months 2, 4, 6, 12, 18, 24. If any blood or marrow samples are positive for DNA, further aspirates will be performed after two years.

4.5 Considerations/Evaluations Upon Premature Withdrawal

4.51 Discontinued patients (see Section 7.0) who have not received transduced cells will not be followed after they are discharged.

4.52 Patients who are discontinued or withdraw before completing all cycles, but have completed at least 1 cycle (patient was reinfused with transduced cells, see Section 7.0) will be asked to return to the clinic to be evaluated according to sections 4.3 and 4.4.

4.6 Autopsy Request: All participants in the study will be informed that an autopsy will be requested to determine the extent of gene transfer into important organs such as marrow, liver, and spleen.

5.0 STUDY TREATMENT

(For additional information see Appendix V and VI)

5.1 Justification of Dosages

It is estimated that the ratio of total bone marrow cells to stem cells is $1:10^5$. With this assumption, then 10^4 to 10^5 stem cells will be exposed to the Gc vector from each patient. If the transduction efficiency is as low as 1%, stem cell transduction should still be detectable in patients. Preclinical studies imply that the transduction frequency into progenitor cells may be as high as 50%. If stem cell transfer is affirmed, conditions for bone marrow or peripheral stem cell collection and transduction will be further optimized.

5.2 Formulation, Packaging, and Storage

More complete descriptions of these reagents are included in Appendix V. Brief descriptions are listed below:

G-CSF

Granulocyte colony stimulating factor is manufactured by Amgen. G-CSF is known to stimulate granulocytes and their precursors. It is used in this protocol to mobilize peripheral blood stem cells.

SCF

Stem cell factor is manufactured by Amgen. It is thought to act synergistically with other growth factors to promote growth of early hematopoietic cells. It is used in this protocol to increase the overall rate of gene transfer into the earliest progenitor cells to improve the efficiency of gene transfer.

IL-3

Interleukin-3 is manufactured by Sandoz. It acts synergistically with a variety of growth factors to stimulate cell division of early marrow cells. It is used in this protocol because its use has been shown to increase the efficiency of gene transfer when retroviral vectors are used.

IL-6

Interleukin-6 is manufactured by Sandoz. Like the previous cytokines, it acts synergistically with other growth factors to stimulate hematopoietic cell division and increase the efficiency of gene transfer.

Gc Retroviral Vector Supernatant

Gc retroviral vector supernatant is manufactured by Genetic Therapy, Inc. It consists of the conditioned medium obtained from Gc producer cells. Gc producer cells have been engineered to produce retroviral vector particles with an amphotropic envelope so that the vector may be transferred into human cells. This vector is referred to as the LG vector in the published literature (20, 25).

5.3 Treatment Procedures

(Refer to section 6.0 for dose modification and toxicity management)

5.31 Harvest of Hematopoietic Stem Cells:

5.311 Peripheral Blood Stem Cell Harvest:

Peripheral blood stem cells will be harvested after mobilization with the growth factor G-CSF.

Study Days P1-P7: G-CSF will be given at a dose of 10 $\mu\text{g/kg/day}$ subcutaneously for a period of 7 consecutive days. The drug will be given as a single daily injection and the patient may receive acetaminophen or other non-aspirin analgesics for any possible associated fever or bone pain.

Study Days P5-P7: On days 5, 6, and 7 the patient will undergo apheresis procedures for the collection of peripheral blood mononuclear cell fractions with an expected yield of 1×10^8 cells per kilogram body weight.

5.312 Bone Marrow Stem Cell Harvest:

Study Day M1: Bone marrow harvest from the iliac crest region will be performed according to standard bone marrow transplant procedures. Appropriate anesthesia will be used. A marrow harvest will consist of approximately one liter in volume and $> 10^8$ nucleated cells / Kg. Harvested marrow will be filtered through the collection kit filters to remove large particulate matter including bony fragments. Further processing of the marrow will be done to enrich for nucleated cells.

5.32 Separation of CD34+ Population of Hematopoietic Cells:

All the cells resulting from the marrow harvest or the peripheral blood stem cell harvest will be processed to remove contaminating red cells. The enrichment for CD34+ cells will be performed using the CellPro Stem Cell Separator. This procedure involves the incubation of the cells with a biotinylated murine anti-human CD34 antibody 12.8, washing the cells, and passing them over a avidin Biogel column. The cells adhering to the column will be collected after mechanical dislodgement. After enrichment a sample of CD34+ cells will be saved for further analysis and to use as pre-gene therapy controls for colony-forming and other assays.

5.33 Transduction and Transplantation Procedures:

The retroviral vector supernatant produced in this study will be produced by Genetic Therapy, Inc. (GTI). GTI has produced the retroviral vector supernatant utilized in most of the gene transfer/therapy studies performed. The vector supernatant used in this protocol will be produced and quality control tested the same as has been done for other retroviral vector supernatants.

5.331 Transduction Procedure

Study Days M1-M3, P5-P10: After removal of aliquots of enriched cells for studies, the remainder of the CD34-enriched population of cells will be transduced with the retroviral vector. Both the marrow-derived and peripheral blood-derived cells will be transduced under the same conditions. The separated CD34-enriched cells will be cultured ($1 \times 10^5/\text{ml}$) in vector supernatant with IL-3, IL-6, and SCF and $4 \mu\text{g/ml}$ protamine sulfate for a period of 72 hours. Every 24 hours the cells will be spun down and resuspended in fresh supernatant, growth factors, and protamine. After the period in culture, the cells will be washed and samples taken for analysis (gene transfer efficiency, GC enzyme activity, colony-forming ability, and replication competent retroviral assays). The remaining cells will be resuspended in heparinized medium for reinfusion. Included in Appendix VII is a sample certificate of the tests that will be performed on each hematopoietic stem cell sample prepared for reinfusion into the patient.

5.332 Reinfusion of Transduced Cells

Study Days M4, P8-P10: The transduced cells will be reinfused into the patient over a period of two hours or less. Premedication, given 30 min before infusion begins, will consist of 25 mg diphenylhydramine IV and 650 mg acetaminophen given po (100 mg hydrocortisone IV is optional). Patients will be closely monitored during this process and several hours after.

5.333 Post-Infusion Care

Patients will be monitored in the hospital overnight. If no adverse reactions are observed, the patients will be discharged the next day (Study Days M5, P11) for subsequent out-patient follow-up. Further patients may receive the infusion as an out-patient.

5.334 Frequency of Infusion of Transduced Cells

The first infusion only may be marrow-harvested cells (if the bone marrow harvest method is chosen, the patients will undergo 1 treatment only). However, if the PBMC-harvest method is chosen, patients may receive up to four cycles (or receive PBMC-transduced cells four times). The cycles will be no more frequent than every 2 months.

5.4 Hazards and Discomforts

This gene transfer protocol would be classified as offering no guarantee of direct benefit to patients enrolled on it, but posing little risk. Information obtained from this pilot gene transfer study could benefit future patients with Gaucher disease, and could possibly allow patients in this study to eventually reduce or discontinue Ceridase enzyme replacement therapy. None of the over 50 patients that have thus far received retrovirally-transduced bone marrow, peripheral blood or tumor infiltrating cells have had any adverse events noted that could be attributed to the gene transfer procedure.

If marrow cells are obtained for transduction, patients will be exposed to the minimal risks associated with the procedure. These risks include the risks associated with the anesthesia used during the marrow harvest. Other risks include infection and bleeding. Pain from the multiple marrow aspirates required to harvest the necessary volume of marrow cells desired can be a problem but is normally responsive to mild-moderate analgesics. Occasional patients may require a blood transfusion after marrow harvest.

Patients will be exposed to the minimal hazards, including bleeding complications and infection, associated with apheresis catheter placement each cycle. They will be treated with G-CSF for 7 days every cycle, and may experience bone pain, fever, or rash, and less commonly reversible elevation of hepatic enzymes, transient depression of platelet numbers, and exacerbation of pre-existing inflammatory conditions. During apheresis the patient may experience postural hypotension and paresthesias.

After the end of the treatment portion of the protocol, patients will be requested to participate in further follow-up to evaluate the duration and extent of GC cDNA transfer and expression. The follow-up will be as an outpatient and will require needle sticks for blood samples, liver-spleen sizing, and additional bone marrow aspirates. As the patient is involved in a gene therapy trial, a regulatory committee has also requested that all patients be followed periodically for the rest of their lives for detection of side-effects from the gene transfer; this request includes that the patient be aware that an autopsy would be requested when the patient dies to measure for possible side-effects including the presence and extent of gene transfer.

Patient Costs: At CHLA, the costs for hospitalization, bone marrow harvest, and any laboratory tests which are performed for study evaluation will be borne by the Division of Research Immunology/Bone Marrow Transplantation. Neither the subject, nor their third party payor will be charged for any of the costs which are part of the experimental investigations. The patients or their third party payor will continue to be responsible for costs of all routine medical care, including Ceredase, if the patient is receiving it.

6.0 TREATMENT MODIFICATION AND DISCONTINUATION ACCORDING TO LEVEL OF TOXICITY

Appendix II is the Toxicity Grading Table and Appendix III is the Adverse Experiences Reporting Guidelines.

The GTI Table for Grading Severity of Adverse Experience (Appendix II) will be used to achieve consistency in response to drug/treatment toxicities. Toxicities will be graded on a 1 - 4 grading scale. If a toxicity is experienced, the treatment level or dose will be modified (if applicable) as outlined below according the grade of toxicity observed.

6.1 Treatment Modification According to Level of Toxicity (General)

- For any Grades 1 - 2 toxicity, there will be no dose modification. However, repeat tests to confirm values within 72 hours may be required.
- Any Grade 3 toxicity, thought to be related to G-CSF, will necessitate a 50% dosage reduction. If a Grade 3 toxicity persists for seven days or advances to Grade 4 toxicity, G-CSF will be discontinued and the patient will not re-start the medication. The patient will continue to have apheresis as if G-CSF were administered for the current cycle only. The patient will receive no further G-CSF and therefore no further cycles of peripheral stem harvest will be attempted under this protocol.
- For Grade 3 toxicity not related to G-CSF, the investigators and sponsor will discuss the various options available. The appropriate action relative to the patient with a Grade 3 toxicity will be evaluated. If appropriate, a decision of whether the study should be continued will be recommended (see Section 7.2). Since no toxicities have been seen in other gene transfer protocols, precedence for the appropriate action is not available and will need to be decided on a case-by-case basis.

6.11 Management of Specific Toxicities

Adverse reactions to apheresis: Reactions are rare, and include anxiety, vasovagal reactions, and cutaneous paresthesias related to citrate-induced hypocalcemia. Sedation, volume repletion, and slowing of the infusion will correct these problems. Bleeding and infection at the line insertion site are potential complications of apheresis catheter placement.

6.2 Symptomatic Therapy for Toxicity

Symptomatic therapy such as analgesics or other helpful therapy may be administered if deemed necessary by the participating investigators. All medications administered will be recorded in the appropriate Case Report Form.

6.3 Replacement Strategy for Discontinued Patients

Discontinued patients that have received gene-modified cells will not be replaced in this study but the discontinued patient will still continue to be followed as previously described in Section 4.4.

7.0 CRITERIA FOR DISCONTINUATION/WITHDRAWAL

7.1 Criteria for Treatment Discontinuation

Participation in this study can be discontinued for any of the following reasons listed below:

- o At the Patient's request: Any patient who wishes to withdraw from the study may do so but will be counselled that long-term follow-up for the effects of gene therapy are strongly desired by the medical facility and federal regulatory groups.
- o A major, unexpected, or life-threatening toxicity requiring that treatment be discontinued
- o Generalized impairment or mental incompetence which would relinquish the patient unable to understand his/her participation in the study.
- o If, in the Investigator's medical judgment, further participation would be injurious to the subject's health or well-being.
- o Administrative reasons, such as subject non-compliance or a major protocol violation.
- o Pregnancy
- o In addition, if at any time during the study a patient is observed to have a level of glucocerebrosidase enzyme equivalent to that of a heterozygote (carrier) individual, the patient will not receive additional cycles of therapy and will be followed to determine the extent and duration of the observed enzyme levels.

An explanation will be recorded for any patient that is taken off treatment.

7.2 Criteria for Study Discontinuation

Patients should understand that once they have completed at least one cycle of therapy, they will be asked to be followed long-term. They can stop study treatment at any time (see section 7.1), however they will still be considered on study until the study has been terminated.

The study can be discontinued only with the understanding of the investigators, sponsor, RAC and the FDA.

8.0 ADVERSE EXPERIENCES

Appendix III is Adverse Experience Reporting (AERs) Guidelines

Depending on the extent of the adverse experience, adverse events should be reported by each site investigator to their local Institutional Review Board (IRB), Institutional BioSafety Committee (IBC) and to Genetic Therapy, Inc. (sponsoring company) as specified in Appendix III.

Adverse reactions or deaths requiring immediate reporting should be made by telephone to (301) 590 2626 (available 24 hours a day, recorder after working hours).

The sponsor, Genetic Therapy, Inc. will take the responsibility of informing and preparing reports for the Recombinant Advisory Committee (RAC) through the NIH Office of Recombinant DNA Activities (ORDA) and to the FDA.

HHS Guidelines for Research Involving Recombinant DNA Molecules: It is the responsibility of the institution and those associated with it to adhere to the intent of the *Guidelines* as well as to their specifics.

9.0 EVALUATION OF RESPONSE AND STUDY ENDPOINTS

9.1 Response Criteria

The study is designed primarily to determine if gene-altered marrow cells can be harvested and readministered to a patient without toxicity. Efficacy is measured in the percentage of various hematopoietic cell populations which contain the transferred gene and the extent to which the transferred gene is responsible for enzyme expression. The primary time point to be used for these determinations is six months after the last gene transfer treatment, although all time points will also be evaluated.

No Response - Less than 0.1 % of the circulating mononuclear cells contain the retroviral vector. No RNA signal from the retroviral vector is detected at any time during the study.

Incomplete Response

- A. Less than 0.1 % of the circulating mononuclear cells contain the retroviral vector. An RNA signal is detected from the retroviral vector at any time during the study.
- B. More than 0.1 % of the circulating mononuclear cells contain the retroviral vector. No RNA signal is detected from the retroviral vector at any time during the study.

Partial Response - More than 0.1% but less than 2% of the circulating mononuclear cells contain the retroviral vector. An RNA signal is detected from the retroviral vector at any time during the study.

Complete Response - More than 2% of the circulating mononuclear cells contain the retroviral vector. An RNA signal is detected from the retroviral vector at any time during the study.

Depending upon the results obtained from this study, alternative vectors and/or treatment schema will be used in future studies to try to increase the extent of glucocerebrosidase expression in Gaucher disease patients.

Toxicity measurements will be analyzed in conjunction with the clinical stage of disease, and other study parameters.

9.2 Study Endpoints

- Development of high grade toxicities or intolerances.
- Expression of GC enzyme levels equivalent to normal patients

10.0 DOCUMENTATION, RECORD KEEPING, CRFs

The investigator must maintain adequate records so that the conduct of the study can be fully documented and monitored.

Copies of protocols, case report forms (CRFs), test result originals, all product accountability records, correspondence, patient informed consent, and any other documents relevant to the conduct of the study must be kept on file by the investigator for (five) years after all investigational use of product is discontinued and the FDA is so notified or until five years after a Product Licensing Application (PLA/ELA) is approved. Study documents should not be destroyed without prior written agreement between GTI and the study investigator. The Study Monitor should be notified if the investigator wishes to assign the study records to another party, or move them to another location.

The investigator will supply the sponsor (GTI) on request with any required background data from all patient records and related documents. This may become necessary if the CRFs are illegible or when errors in data transcription are questionable. For FDA inspections, it is also necessary to have access to complete study patient records, provided that patient confidentiality is maintained.

The investigator should obtain a separate release of medical information form to be signed by the study patient in order to facilitate access to the patient's medical records should the patient be hospitalized at an institution with which the study investigator is not associated.

A record must be kept of all patients considered (and screened) for the study and subsequently deemed ineligible. The reason for ineligibility must be recorded.

11.0 DATA COLLECTION AND STUDY MONITORING

11.1 Data Collection

Case Report Forms (CRFs) will be available for each patient enrolled in the study. Study participants must NOT be identified by name on any study documents. Patients will be identified by a patient identification number (PIN).

11.2 Study Monitoring

It is understood that the GTI-designated person responsible for study monitoring of patient data will be permitted to contact and visit the investigator at regular intervals and will be allowed, on request, to review study documents such as consent forms, drug distribution forms, case report forms, and pertinent hospital or clinic records for confirmation of the study data provided that patient confidentiality is maintained in accord with local requirements. It will be the monitor's responsibility to verify the adherence to the protocol and the completeness, consistency, and accuracy of the data being entered on the CRFs. If local regulations permit, the study monitor should have access to original laboratory test reports and other patient documents (source documentation) needed to verify data entered on the CRFs. The investigator will cooperate with the assigned study monitor to ensure that all problems are resolved.

12.0 BIOSTATISTICAL CONSIDERATIONS

Summaries for the following parameters will be compiled at six months and one year:

- ▶ Percentage of cells containing the GC vector (extent of engraftment)
- ▶ Mean glucocerebrosidase levels in study participants
- ▶ Safety

13.0 ETHICAL CONSIDERATIONS

The investigator will ensure that the study is conducted in full conformance with the FDA standards for human research.

13.1 Informed Consent

All study participants must sign an informed consent. The investigators will inform all subjects as to the nature, aims, duration, potential hazards, and procedures to be performed during the study and that his or her medical records may be reviewed by the sponsors or the FDA. This protocol must receive approval by the Institutional Review Board of all participating sites prior to implementation. The investigator must also explain that the patients are completely free to refuse to enter the study or to withdraw from it at any time. The protocol will be discussed in detail with all potentially eligible

patients. All revisions of the protocol must be reflected in the consent form and reviewed by the IRB.

Appendix VI are the Patient Informed Consents (attachments)

13.2 Patient Confidentiality

All reports and patient samples will be identified only by a coded number to maintain patient confidentiality. All records will be kept confidential to the extent permitted by law. The investigator should keep a separate log of patients' codes, names, and addresses. Documents which identify the patient by name (informed consent) should be kept in strict confidence.

14.0 **CRITERIA AND PROCEDURES FOR PROTOCOL MODIFICATION AND STUDY TERMINATION**

Modifications which may effect the safety of the study patient, or which may alter the scope of the investigation, the scientific quality of the study, the study design, dosages, duration of therapy, patient assessments (added evaluation that poses potential risk or inconvenience to the patient), number of patients, and patient eligibility criteria, may be made only after appropriate consultation between the sponsor and the investigator.

If the consensus is to revise the current protocol, a formal List of Changes will accompany the amended protocol and these will be submitted to the FDA, the site's IRB, and other committees as required.

Both the sponsor and the investigator reserve the right to terminate the study at any time. If this becomes necessary, appropriate procedures for continuing the long-term follow-up requested by the regulatory agencies will be arranged after review and approval by both parties.

15.0 **PUBLICATION OF RESEARCH FINDINGS/PROTECTION OF COMPANY TRADE SECRETS**

The investigator will agree to inform the sponsor of all study results. Any manuscript, abstract, or presentation will be made available to the sponsor and all participating study investigators for review prior to submission of manuscripts, abstracts, or scientific meetings. This will allow the sponsor to protect proprietary information and to provide comment based on information that may not yet be available to the investigator.

16.0 **BIOHAZARD WARNINGS AND PRECAUTIONS**

Patients will be treated in standard hospital and clinic rooms. No special patient precautions will be taken.

17.0 REFERENCES

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5/7/93

MEDICAL RECORD

CONSENT TO PARTICIPATE IN A CLINICAL RESEARCH STUDY

• Adult Patient or • Parent, for Minor Patient

INSTITUTE: National Heart, Lung and Blood Institute, National Institute of Neurologic Disorders and StrokeSTUDY NUMBER _____ PRINCIPAL INVESTIGATOR: Cynthia E. Dunbar, M.D.
Norman Barton, M.D.STUDY TITLE: Retroviral Mediated Transfer of cDNA for Human Glucocerebrosidase into Hematopoietic Stem Cells of Patients with Gaucher Disease (Peripheral Blood Stem Cell Arm)**INTRODUCTION**

We invite you (or your child) to take part in a research study at the National Institutes of Health. It is important that you read and understand several general principles that apply to all who take part in our studies: (a) taking part in the study is entirely voluntary; (b) personal benefit may not result from taking part in the study, but knowledge may be gained that will benefit others; (c) you may withdraw from the study at any time without penalty or loss of any benefits to which you are otherwise entitled. The nature of the study, the risks, inconveniences, discomforts, and other pertinent information about the study are discussed below. You are urged to discuss any questions you have about this study with the staff members who explain it to you.

BACKGROUND AND PURPOSE

Gaucher Disease is a genetic disorder caused by an abnormality of a certain gene. This gene is responsible for directing the cells in the body to produce an enzyme called glucocerebrosidase (GC). Therefore, patients with Gaucher disease do not have normal activity levels of this enzyme in their body. This lack results in the body accumulating certain waste products, called glucocerebrosides, which can be damaging to the body. These waste products can accumulate in many parts of the body. This process is most severe in the spleen, liver, and bone marrow. Spleen involvement often results in an extremely large spleen; which can cause severe problems, such as low blood counts, bleeding, and inability to fight infections. Bone marrow involvement can cause bone pain, bone deformities, and bone fractures. Liver involvement can lead to liver failure. Other organs besides liver, bone marrow and spleen can be affected in Gaucher disease. The enlarged spleen and liver may make breathing more difficult. The heart can be overworked pumping large amounts of blood to the enlarged organs. The skin can also be affected.

Over the past twenty years, there has been a great deal of research into the treatment of Gaucher disease. One form of treatment is Bone Marrow Transplantation, using bone marrow from a brother or

PATIENT IDENTIFICATION

CONSENT TO PARTICIPATE IN A CLINICAL RESEARCH STUDY

• Adult Patient or • Parent, for Minor Patient

NIH-2514-1 (9-91)

P.A.:09-25-0099

Recombinant DNA Research, Volume 17

File in Section 4: Protocol Consent

MEDICAL RECORD

CONTINUATION SHEET for either:

NIH 2514-1, Consent to Participate In A Clinical Research Study

NIH 2514-2, Minor Patient's Assent to Participate In A Clinical Research Study

STUDY NUMBER: _____ CONTINUATION: page 2 of 9 pages.

sister that does not have Gaucher disease. Bone Marrow Transplantation is often successful in reversing or minimizing the problems associated with Gaucher disease. However, the majority of patients will not have an appropriate family donor. In addition there are many serious health risks associated with bone marrow transplantation. Since bone marrow transplantation is often not possible or desirable, alternative treatments have been tested. The best available treatment for Gaucher disease is known as enzyme replacement therapy. Enzyme replacement therapy with "Ceredase", the commercially available product, is effective and safe in most patients, and you may be receiving this therapy at the present time. However, enzyme replacement therapy must continue for the entire life of a Gaucher disease patient, is very expensive, and may not be available to many patients with Gaucher disease.

The purpose of this study is to investigate an alternative experimental form of therapy for Gaucher disease called "gene therapy". We seek to determine if we can use gene therapy procedures to insert the normal gene responsible for producing the missing GC enzyme into immature blood cells of patients with Gaucher disease. Certain cells in the blood and bone marrow called "stem cells" are responsible for producing all the blood cells in the body, such as red blood cells, white blood cells, and platelets. One type of cell produced by these stem cells, called a "macrophage", is the cell that builds up the abnormal waste products in Gaucher disease. If we could insert the normal gene for the missing GC enzyme into the stem cells responsible for making macrophages, then possibly these macrophages would no longer build up the toxic waste products, and some of the problems associated with Gaucher disease could be eliminated.

We will collect bone marrow or blood stem cells from patients with Gaucher disease and treat them in the laboratory with a procedure designed to place the GC gene in the cells. These treated cells will then be placed back in the patient's body. We will then perform a number of tests to determine whether the new gene is present, and whether or not it is working. It is important to realize that at this stage, this experimental procedure is only designed to test the safety of these new procedures. There is only a remote chance that this procedure will result in any direct benefit to you, though it is possible that the results of this study will lead to a form of "gene therapy" which may benefit you or other patients in the future.

PATIENT IDENTIFICATION

CONTINUATION SHEET for either:

NIH-2514-1 (10-84)

NIH-2514-2 (10-84)

STUDY NUMBER: _____ CONTINUATION: page 3 of 9 pages.

Pre-Study Testing: Prior to being entered into this study you will undergo blood tests, bone marrow aspiration and biopsy, X-ray and other imaging studies, and physical examination to determine the status of your Gaucher disease and of your liver, kidneys and bone marrow. You will have 2-4 tablespoons of blood drawn. You will sign a separate consent form for the bone marrow aspiration and biopsy, which will be done with local anesthesia and has minimal risks, including pain, bleeding and infection at the biopsy site.

Collection of Peripheral Blood Stem Cells:

G-CSF Administration: To increase the number of immature stem cells in the blood, the drug G-CSF (granulocyte colony-stimulating factor) will be administered either as an outpatient in the hematology clinic or as an inpatient on the hematology ward at the Clinical Center. This drug stimulates the bone marrow to release stem cells into the blood, where they can be more easily collected. G-CSF will be given as a daily injection under the skin for 7 days. G-CSF may cause fever, flu-like symptoms, muscle aches, bone pain, rash, redness at the injection site, decrease in platelet count, and temporary worsening of skin conditions such as psoriasis and eczema. You will be treated for any of these symptoms if they occur. These symptoms disappear immediately after discontinuation of the drug. It is also possible that G-CSF could temporarily worsen the bone symptoms caused by your Gaucher disease. If there is any evidence for these problems, the G-CSF would be stopped immediately and you would be taken off protocol.

Central Line Placement: On the 4th or 5th day after starting G-CSF, you will have a special intravenous line called a "central line" inserted into a large vein in your groin, neck, or chest. This will be done using local anesthesia by a line insertion specialist. You will sign a separate consent for this procedure. This large intravenous line is necessary for collection of stem cells from your blood. You may have mild pain and bruising at the insertion site. Rarely, infections or more serious bleeding develop at the insertion site, which can be treated by removal of the line, antibiotics, or pressure to the site. This line will be removed on day 7, after completion of stem cell collection.

Apheresis: On days 5, 6 and 7 after beginning G-CSF, you will undergo a procedure called "apheresis" to collect the stem cells in your blood. You will be hooked up to an apheresis machine via one lumen (opening) of your central line, and blood will slowly flow through the machine for 1-3 hours. The machine will separate out

MEDICAL RECORD	CONTINUATION SHEET for either: NIH 2514-1, Consent to Participate In A Clinical Research Study NIH 2514-2, Minor Patient's Assent to Participate In A Clinical Research Study
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and save a fraction of your blood containing stem cells, and continuously return red blood cells, platelets, and plasma to you through the second lumen of your central line. Apheresis has very few side effects, but some patients experience light-headedness, nausea, or tingling in the fingers and toes. These symptoms can be corrected by slowing down the blood flow through the apheresis machine. The G-CSF will be discontinued on day 7. Repeated apheresis can make patients anemic. We will follow your blood counts carefully, and discontinue apheresis procedures before you become anemic enough to require transfusion, but there is a very small possibility that you could require a blood transfusion as a result of this protocol. You will not be entered into this protocol if you are already severely anemic.

Stem Cell Purification: The cells collected by apheresis will be further purified after harvest in the laboratory by a procedure called "CD34 selection." The cells will be exposed to an antibody (protein) that sticks specifically to the surface of immature cells. The cells will then be run through a special column that separates the immature cells coated with antibody from the more mature cells. This antibody and column procedure is experimental, but has been safely used to purify stem cells from the bone marrow and blood of over 50 patients without any known side effects.

Gene Transfer: The purified stem cells will be treated in the laboratory for 3-4 days in the presence of a "vector" designed to place the GC gene in the cells' genetic material (chromosomes). Once the GC gene is in the cell, it is permanent, and the cell will pass the GC gene onto all daughter cells derived from that cell. The vector is a mouse virus which has been altered so it cannot reproduce itself. No side effects have been seen in over 50 patients that have received cells treated with this type of vector, although no patients prior to this study have received cells treated with the GC vector.

This is a very new procedure, and there may be risks that have not yet been discovered. Theoretically, there may be an increased risk of cancer in cells treated with vectors, however, this risk is minimized by making sure that the vector used can not reproduce itself once it is inside patient cells. Animals and patients receiving cells treated with this type of vector have not developed new cancers attributable to the vector.

Your cells will be grown in several growth factors called IL-3, IL-

PATIENT IDENTIFICATION Recombinant DNA Research, Volume 17	CONTINUATION SHEET for either: NIH-2514-1 (10-84) NIH-2514-2 (10-84) P.A. 09-25-0099 [825]
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-6 and SCF during vector exposure in the laboratory. These are necessary to keep the stem cells alive and to increase the chance that the cells will take up the vector. These growth factors are experimental drugs, but they have been given to patients without serious side effects. Your cells will be washed thoroughly before giving them back to you, and we have not been able to detect any growth factors remaining in the solution containing the stem cells that will be reinfused.

Stem Cell Reinfusion: After 3-4 days in culture with vector and growth factors, the cells will be collected, washed, and given back to you intravenously. You will receive three daily infusions of cells on days 8, 9 and 10 after beginning G-CSF. The infusions will take 10-30 minutes, and you will be monitored closely for side effects during and after the infusions. We do not expect any side effects, but an allergic reaction is theoretically possible, and you would be treated for it occurred. It is also possible that the cells could become infected with bacteria or fungus during the 3 day growth period in the laboratory. If there is any evidence for infection of the cells with unwanted agents, the cells will not be returned to you, but there is a remote chance that you could acquire a bacterial or fungal infection from the reinfused cells.

After completion of the third infusion, you will be able to return home. You will return to NIH every 1-4 months for follow-up and to repeat the whole stem cell collection and gene transfer procedure for a total of four times.

Follow-up: You will be followed as an outpatient approximately every month for at least one year. We will perform a number of blood tests to determine whether the gene is present in your blood cells, and whether it is working to produce the normal GC enzyme. We will also perform tests to make sure that the gene transfer procedure has been safe. The amount of blood drawn will be approximately two-four tablespoons every month. We may also request permission to perform a bone marrow aspiration tests up to six times over a two year period. X-rays or scans may be performed as necessary for the routine clinical care of your Gaucher disease, and to assess whether the gene transfer procedure has had any effect on your clinical condition.

Since this study involves a very new approach to patients with Gaucher disease, it will be important to maintain contact with you for the rest of your life, so that we can monitor your overall progress and monitor for any possible long term side effects. If

UDY NUMBER: _____ CONTINUATION: page 7 of 9 pages.

the GC gene is successfully transferred into stem cells, your body would contain cells carrying the transferred gene for life. We would continue to see you in our clinic yearly for at least five years. In the event of your death, we would request an autopsy for the purpose of determining whether the gene has been transferred into important organs, such as the liver or spleen.

It is important to note that participation in this study will in no way alter any of the standard care you may be receiving. If you are currently receiving enzyme replacement therapy, this should continue to be administered at the same dose through your primary physician. There will be no charge to you or your insurance carrier for any of the tests, procedures, or hospital stays, associated with this study. You will continue to be responsible for the costs of standard care, including enzyme replacement. You may withdraw from this study at any time, and we would continue to follow you as described above without prejudice. If you remain on the study for the entire year, you are then free to explore any other treatment options, standard or experimental.

Potential Benefits: We do not know if there will be any direct benefits to you from participating in this study. The major goal of this study is to determine if this gene transfer approach is safe and feasible in Gaucher patients. However, there may be future benefits to you and other patients with Gaucher disease. It is possible that the results of this study will lead to a new form of therapy for Gaucher disease for you and other patients with Gaucher disease, or other genetic disorders.

Potential Risks: The specific risks associated with each component of this protocol were described above. In any new form of therapy, there may be risks which are unknown or not anticipated. Since the vector does not replicate inside the body, we do not anticipate that it can alter your sperm/ova, and therefore we would not anticipate that this treatment could adversely affect your offspring. Participation in this study will not lower the risk of passing Gaucher disease to your offspring.

Woman of child-bearing potential are advised to use an effective method of birth control until the safety of this therapy has been fully established. Men are advised to use barrier-protection while participating in this study. Females must have had a negative pregnancy test within 14 days prior to dosing. If you should become pregnant while on this study you must inform the investigator immediately.

As part of your participation in this study, it will be necessary to test your blood for the presence of antibodies to the Human Immunodeficiency Virus (HIV), the virus that causes Acquired Immune Deficiency Syndrome (AIDS). In order to perform the test, a small amount of blood (approximately 2 teaspoons) will be withdrawn from one of your arms with a needle. You may experience some slight discomfort at the needle entry site and there may be some bruising. In addition, there is a very small risk of your fainting or of infection at the needle entry site. If your test results are found to be positive, or if you are otherwise diagnosed as having AIDS, you should be aware of the following Clinical Center HIV Testing Policy:

1. Your physician will notify you promptly of the HIV test results.
2. Your physician and/or the Clinical Center HIV counselor will offer you, and any current and/or ongoing sexual partner(s) (spouses are generally considered to be current or ongoing sexual partners) or needle-sharing partner(s) you identify, information on the meaning of the test results and how to prevent the spread of the infection.
3. Because the virus may be transmitted in several ways, it is important that you inform sexual and/or needle-sharing partner(s) that any, or all, of them may have been exposed to the HIV virus and encourage them to be tested. If you request it, staff at the Clinical Center will assist you in notifying your partner(s) and arrange counseling for them through an HIV counselor.
4. The results of your HIV test and/or documentation of the diagnosis of AIDS will become a part of your Clinical Center medical record and, as such, will be protected from unauthorized disclosure by the Federal Privacy Act of 1974. In general, access to your medical record will be restricted to those health care professionals directly involved in your care or in the conduct of ongoing biomedical research, and information is not usually released to other third parties without your permission or that of your designated representative. However, there are some particular routine uses of such information of which you should be aware.
 - a. If you are unwilling or unable to notify your partner(s), the Clinical Center is responsible for attempting to contact and inform them of their possible exposure to the virus. Reasonable attempts will be made to protect your identity including withholding your name when notifying any partner(s) of their possible exposure. Some notification or counseling of current and/or ongoing partners may be carried out through arrangements with, or referral to, local public health agencies.
 - b. A summary of your care at the Clinical Center will be sent to the physician who referred you here for treatment.
 - c. The Clinical Center may report certain communicable diseases, including AIDS and symptomatic HIV infection, to appropriate State and Federal government agencies.

If you have any questions regarding the HIV testing or the information provided above, you are encouraged to discuss them with your physician and/or a Clinical Center HIV counselor (496-2381).

Patient Identification

INCLUSION OF HIV TESTING IN CONSENT TO
PARTICIPATE IN A CLINICAL RESEARCH STUDY
NIH-2514-3 (2-90)

P.A. 09-25-0099

File in Section 4: Protocol Consent

STUDY NUMBER: _____

OTHER PERTINENT INFORMATION

1. Confidentiality. When results of a study such as this are reported in medical journals or at meetings, the identification of those taking part is withheld. Medical records of National Institutes of Health or Clinical Center patients are maintained according to current legal requirements, and are made available for review, as required by the Food and Drug Administration or other authorized users, only under the guidelines established by the Federal Privacy Act.
2. Policy Regarding Research-Related Injuries. The Clinical Center will provide short-term medical care for any physical injury resulting from your participation in research here. Neither the National Institutes of Health, Clinical Center nor the Federal government will provide long-term medical care or financial compensation for such injuries, except as may be provided through whatever remedies are normally available under law.
3. Payments. If you are a patient, you are not paid for taking part in National Institute of Health studies. Exceptions for volunteers will be guided by the National Institutes of Health or Clinical Center policies.
4. Problems or Questions. Should any problem or question arise with regard to this study, with regard to your rights as a participant in clinical research, or with regard to any research-related injury, you should contact the principal investigator, Cynthia E. Dunbar, M.D., or these other staff members also involved in this study: Donna Vining, M.D.; Norman Barton, M.D., Ph.D.; Michelle Cottler-Fox, M.D.; Building 10, Room 7C103. Telephone: (301) 496-5093.
National Institutes of Health
Bethesda, Maryland 20205
5. Consent Document. It is suggested that you retain a copy of this document for your later reference and personal records.

COMPLETE APPROPRIATE ITEM BELOW, A or B:

A. Adult Patient's Consent.

I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby consent to take part in this study.

Signature of Adult Patient & Date Signed

B. Parent's Permission for Minor Patient.

I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby give permission for my child to take part in this study.

(Attach NIH 2514-2, Minor's Assent, if applicable.)

Signature of Parent(s) & Date Signed_____
(If Other Than Parent, Specify Relationship)_____
Signature of Investigator & Date Signed_____
Signature of Witness & Date Signed

PATIENT IDENTIFICATION

CONSENT TO PARTICIPATE IN A CLINICAL RESEARCH STUDY (Continuation Sheet)

• Adult Patient or • Parent, for Minor Patient

NIH-2514-1 (9-91)

P A 09-25-0099

File in Section 4 Protocol Consent

5/7/93

MEDICAL RECORD

CONSENT TO PARTICIPATE IN A CLINICAL RESEARCH STUDY

• Adult Patient or • Parent, for Minor Patient

INSTITUTE: National Heart, Lung and Blood Institute, National Institute of Neurologic Disorders and Stroke

STUDY NUMBER _____ PRINCIPAL INVESTIGATOR: Cynthia E. Dunbar, M.D.
Norman Barton, M.D.

STUDY TITLE: Retroviral Mediated Transfer of the cDNA for Human Glucocerebrosidase into Hematopoietic Stem Cells of Patients with Gaucher Disease (Bone Marrow Stem Cell Arm)

INTRODUCTION

We invite you (or your child) to take part in a research study at the National Institutes of Health. It is important that you read and understand several general principles that apply to all who take part in our studies: (a) taking part in the study is entirely voluntary; (b) personal benefit may not result from taking part in the study, but knowledge may be gained that will benefit others; (c) you may withdraw from the study at any time without penalty or loss of any benefits to which you are otherwise entitled. The nature of the study, the risks, inconveniences, discomforts, and other pertinent information about the study are discussed below. You are urged to discuss any questions you have about this study with the staff members who explain it to you.

BACKGROUND AND PURPOSE

Gaucher Disease is a genetic disorder caused by an abnormality of a certain gene. This gene is responsible for directing the cells in the body to produce an enzyme called glucocerebrosidase (GC). Therefore, patients with Gaucher disease do not have normal activity levels of this enzyme in their body. This lack results in the body accumulating certain waste products, called glucocerebrosides, which can be damaging to the body. These waste products can accumulate in many parts of the body. This process is most severe in the spleen, liver, and bone marrow. Spleen involvement often results in an extremely large spleen, which can cause severe problems, such as low blood counts, bleeding, and inability to fight infections. Bone marrow involvement can cause bone pain, bone deformities, and bone fractures. Liver involvement can lead to liver failure. Other organs besides liver, bone marrow and spleen can be affected in Gaucher disease. The enlarged spleen and liver may make breathing more difficult. The heart can be overworked pumping large amounts of blood to the enlarged organs. The skin can also be affected.

Over the past twenty years, there has been a great deal of research into the treatment of Gaucher disease. One form of treatment is Bone Marrow Transplantation, using bone marrow from a brother or sister that does not have Gaucher disease. Bone Marrow

PATIENT IDENTIFICATION

CONSENT TO PARTICIPATE IN A CLINICAL RESEARCH STUDY

• Adult Patient or • Parent, for Minor Patient

NIH-2514-1 (9-91)

P.A. 09-25-0093

Recombinant DNA Research, Volume 17

MEDICAL RECORD	CONTINUATION SHEET for either: NIH 2514-1, Consent to Participate In A Clinical Research Study NIH 2514-2, Minor Patient's Assent to Participate In A Clinical Research Study
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STUDY NUMBER: _____ CONTINUATION: page 2 of 8 pages.

Transplantation is often successful in reversing or minimizing the problems associated with Gaucher disease. However, the majority of patients will not have an appropriate family donor. In addition there are many serious health risks associated with bone marrow transplantation. Since bone marrow transplantation is often not possible or desirable, alternative treatments have been tested. The best available treatment for Gaucher disease is known as enzyme replacement therapy. Enzyme replacement therapy with "Ceredase", the commercially available product, is effective and safe in most patients, and you may be receiving this therapy at the present time. However, enzyme replacement therapy must continue for the entire life of a Gaucher disease patient, is very expensive, and may not be available to many patients with Gaucher disease.

The purpose of this study is to investigate an alternative experimental form of therapy for Gaucher disease called "gene therapy". We seek to determine if we can use gene therapy procedures to insert the normal gene responsible for producing the missing GC enzyme into immature blood cells of patients with Gaucher disease. Certain cells in the blood and bone marrow called "stem cells" are responsible for producing all the blood cells in the body, such as red blood cells, white blood cells, and platelets. One type of cell produced by these stem cells, called a "macrophage", is the cell that builds up the abnormal waste products in Gaucher disease. If we could insert the normal gene for the missing GC enzyme into the stem cells responsible for making macrophages, then possibly these macrophages would no longer build up the toxic waste products, and some of the problems associated with Gaucher disease could be eliminated.

We will collect bone marrow or blood stem cells from patients with Gaucher disease and treat them in the laboratory with a procedure designed to place the GC gene in the cells. These treated cells will then be placed back in the patient's body. We will then perform a number of tests to determine whether the new gene is present, and whether or not it is working. It is important to realize that at this stage, this experimental procedure is only designed to test the safety of these new procedures. There is only a remote chance that this procedure will result in any direct benefit to you, though it is possible that the results of this study will lead to a form of "gene therapy" which may benefit you or other patients in the future.

Pre-Study Testing: Prior to being entered into this study you will undergo blood tests, bone marrow aspiration and biopsy, X-ray and

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other imaging studies, and physical examination to determine the status of your Gaucher disease and of your liver, kidneys and bone marrow. You will have 2-4 tablespoons of blood drawn. You will sign a separate consent form for the bone marrow aspiration and biopsy, which will be done with local anesthesia and has minimal risks, including pain, bleeding and infection at the biopsy site.

Collection of Bone Marrow: The first part of the protocol involves removing a portion of your bone marrow. You will be brought to the operating room and be placed under general anesthesia. The doctors will then remove about one liter of bone marrow from the area on the back of your hip bone known as the posterior iliac crest. This is done with hollow needles that are placed through the skin, and then through the outer surface of the bone. The harvest takes one-two hours. Risks include bleeding or infection. You will need to stay in the hospital the night after the harvest, and you will experience pain over your hip bones. You will receive pain medication to relieve this pain if necessary. There is a small possibility that the bone marrow harvest will make you anemic enough to require a blood transfusion. You will not be entered onto this protocol if you are already severely anemic, and we will do everything possible to prevent the need for a transfusion of blood from another person, including returning the red blood cells collected during the bone marrow harvest to you.

Stem Cell Purification: The bone marrow cells will be further purified after harvest in the laboratory by a procedure called "CD34 selection." The cells will be exposed to an antibody (protein) that sticks specifically to the surface of immature cells. The cells will then be run through a special column that separates the immature cells coated with antibody from the more mature cells. This antibody and column procedure is experimental, but has been safely used to purify stem cells from the bone marrow and blood of over 50 patients without any known side effects.

Gene Transfer: The purified stem cells will be treated in the laboratory for 3-4 days in the presence of a "vector" designed to place the GC gene in the cells' genetic material (chromosomes). Once the GC gene is in the cell, it is permanent, and the cell will pass the GC gene onto all daughter cells derived from that cell. The vector is a mouse virus which has been altered so it cannot reproduce itself. No side effects have been seen in over 50 patients that have received cells treated with this type of vector, although no patients prior to this study have received cells treated with the GC vector.

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This is a very new procedure, and there may be risks that have not yet been discovered. Theoretically, there may be an increased risk of cancer in cells treated with vectors, however, this risk is minimized by making sure that the vector used can not reproduce itself once it is inside patient cells. Animals and patients receiving cells treated with this type of vector have not developed new cancers attributable to the vector.

Your cells will be grown in several growth factors called IL-3, IL-6 and SCF during vector exposure in the laboratory. These are necessary to keep the stem cells alive and to increase the chance that the cells will take up the vector. These growth factors are experimental drugs, but they have been given to patients without serious side effects. Your cells will be washed thoroughly before giving them back to you, and we have not been able to detect any growth factors remaining in the solution containing the stem cells that will be reinfused.

Stem Cell Reinfusion: After 3-4 days in culture with vector and growth factors, the cells will be collected, washed, and given back to you intravenously. You will receive three daily infusions of cells on days 8, 9 and 10 after beginning G-CSF. The infusions will take 10-30 minutes, and you will be monitored closely for side effects during and after the infusions. We do not expect any side effects, but an allergic reaction is theoretically possible, and you would be treated for it occurred. It is also possible that the cells could become infected with bacteria or fungus during the 3 day growth period in the laboratory. If there is any evidence for infection of the cells with unwanted agents, the cells will not be returned to you, but there is a remote chance that you could acquire a bacterial or fungal infection from the reinfused cells.

Follow-up: You will be followed as an outpatient approximately every month for at least one year. We will perform a number of blood tests to determine whether the gene is present in your blood cells, and whether it is working to produce the normal GC enzyme. We will also perform tests to make sure that the gene transfer procedure has been safe. The amount of blood drawn will be approximately two-four tablespoons every month. We may also request permission to perform a bone marrow aspiration tests up to six times over a two year period. X-rays or scans may be performed as necessary for the routine clinical care of your Gaucher disease, and to assess whether the gene transfer procedure has had any effect on your clinical condition.

STUDY NUMBER: _____ CONTINUATION: page 5 of 8 pages

Since this study involves a very new approach to patients with Gaucher disease, it will be important to maintain contact with you for the rest of your life, so that we can monitor your overall progress and monitor for any possible long term side effects. If the GC gene is successfully transferred into stem cells, your body would contain cells carrying the transferred gene for life. We would continue to see you in our clinic yearly for at least five years. In the event of your death, we would request an autopsy for the purpose of determining whether the gene has been transferred into important organs, such as the liver or spleen.

It is important to note that participation in this study will in no way alter any of the standard care you may be receiving. If you are currently receiving enzyme replacement therapy, this should continue to be administered at the same dose through your primary physician. There will be no charge to you or your insurance carrier for any of the tests, procedures, or hospital stays, associated with this study. You will continue to be responsible for the costs of standard care, including enzyme replacement. You may withdraw from this study at any time; and we would continue to follow you as described above without prejudice. If you remain on the study for the entire year, you are then free to explore any other treatment options, standard or experimental.

Potential Benefits: We do not know if there will be any direct benefits to you from participating in this study. The major goal of this study is to determine if this gene transfer approach is safe and feasible in Gaucher patients. However, there may be future benefits to you and other patients with Gaucher disease. It is possible that the results of this study will lead to a new form of therapy for Gaucher disease for you and other patients with Gaucher disease, or other genetic disorders.

Potential Risks: The specific risks associated with each component of this protocol were described above. In any new form of therapy, there may be risks which are unknown or not anticipated. Since the vector does not replicate inside the body, we do not anticipate that it can alter your sperm/ova; and therefore we would not anticipate that this treatment could adversely affect your offspring. Participation in this study will not lower the risk of passing Gaucher disease to your offspring.

Woman of child-bearing potential are advised to use an effective method of birth control until the safety of this therapy has been fully established. Men are advised to use barrier-protection while

MEDICAL RECORD**CONTINUATION SHEET for either:**

NIH 2514-1, Consent to Participate In A Clinical Research Study

NIH 2514-2, Minor Patient's Assent to Participate In A Clinical Research Study

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participating in this study. Females must have had a negative pregnancy test within 14 days prior to dosing. If you should become pregnant while on this study you must inform the investigator immediately.

PATIENT IDENTIFICATION**CONTINUATION SHEET for either:**

NIH-2514-1 (10-84)

NIH-2514-2 (10-84)

P.A.: 09-25-0099

[835]

As part of your participation in this study, it will be necessary to test your blood for the presence of antibodies to the Human Immunodeficiency Virus (HIV), the virus that causes Acquired Immune Deficiency Syndrome (AIDS). In order to perform the test, a small amount of blood (approximately 2 teaspoons) will be withdrawn from one of your arms with a needle. You may experience some slight discomfort at the needle entry site and there may be some bruising. In addition, there is a very small risk of your fainting or of infection at the needle entry site. If your test results are found to be positive, or if you are otherwise diagnosed as having AIDS, you should be aware of the following Clinical Center HIV Testing Policy:

1. Your physician will notify you promptly of the HIV test results.
2. Your physician and/or the Clinical Center HIV counselor will offer you, and any current and/or ongoing sexual partner(s) (spouses are generally considered to be current or ongoing sexual partners) or needle-sharing partner(s) you identify, information on the meaning of the test results and how to prevent the spread of the infection.
3. Because the virus may be transmitted in several ways, it is important that you inform sexual and/or needle-sharing partner(s) that any, or all, of them may have been exposed to the HIV virus and encourage them to be tested. If you request it, staff at the Clinical Center will assist you in notifying your partner(s) and arrange counseling for them through an HIV counselor.
4. The results of your HIV test and/or documentation of the diagnosis of AIDS will become a part of your Clinical Center medical record and, as such, will be protected from unauthorized disclosure by the Federal Privacy Act of 1974. In general, access to your medical record will be restricted to those health care professionals directly involved in your care or in the conduct of ongoing biomedical research, and information is not usually released to other third parties without your permission or that of your designated representative. However, there are some particular routine uses of such information of which you should be aware.
 - a. If you are unwilling or unable to notify your partner(s), the Clinical Center is responsible for attempting to contact and inform them of their possible exposure to the virus. Reasonable attempts will be made to protect your identity including withholding your name when notifying any partner(s) of their possible exposure. Some notification or counseling of current and/or ongoing partners may be carried out through arrangements with, or referral to, local public health agencies.
 - b. A summary of your care at the Clinical Center will be sent to the physician who referred you here for treatment.
 - c. The Clinical Center may report certain communicable diseases, including AIDS and symptomatic HIV infection, to appropriate State and Federal government agencies.

If you have any questions regarding the HIV testing or the information provided above, you are encouraged to discuss them with your physician and/or a Clinical Center HIV counselor (496-2381).

Patient Identification

INCLUSION OF HIV TESTING IN CONSENT TO
PARTICIPATE IN A CLINICAL RESEARCH STUDY
NIH-2514-3 (2-90)

P.A. 09-25-0099

File in Section 4: Protocol Consent

MEDICAL RECORD	CONSENT TO PARTICIPATE IN A CLINICAL RESEARCH STUDY • Adult Patient or • Parent, for Minor Patient	continuation: page <u>8</u> of <u>8</u> pages
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STUDY NUMBER: _____

OTHER PERTINENT INFORMATION

1. Confidentiality. When results of a study such as this are reported in medical journals or at meetings, the identification of those taking part is withheld. Medical records of National Institutes of Health or Clinical Center patients are maintained according to current legal requirements, and are made available for review, as required by the Food and Drug Administration or other authorized users, only under the guidelines established by the Federal Privacy Act.
2. Policy Regarding Research-Related Injuries. The Clinical Center will provide short-term medical care for any physical injury resulting from your participation in research here. Neither the National Institutes of Health, Clinical Center nor the Federal government will provide long-term medical care or financial compensation for such injuries, except as may be provided through whatever remedies are normally available under law.
3. Payments. If you are a patient, you are not paid for taking part in National Institute of Health studies. Exceptions for volunteers will be guided by the National Institutes of Health or Clinical Center policies.
4. Problems or Questions. Should any problem or question arise with regard to this study, with regard to your rights as a participant in clinical research, or with regard to any research-related injury, you should contact the principal investigator, Cynthia E. Dunbar, M.D., or these other staff members also involved in this study:
Norman Barton, M.D. ; Donna Vining, R.N. ; Michelle Cottler-Fox, M.D. ;
 Building 10, Room 7C103. Telephone: (301) 496-5093
496-1465
 National Institutes of Health
 Bethesda, Maryland 20205
5. Consent Document. It is suggested that you retain a copy of this document for your later reference and personal records.

COMPLETE APPROPRIATE ITEM BELOW, A or B:

A. Adult Patient's Consent.

I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby consent to take part in this study.

 Signature of Adult Patient & Date Signed

B. Parent's Permission for Minor Patient.

I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby give permission for my child to take part in this study.
 (Attach NIH 2514-2, Minor's Assent, if applicable.)

 Signature of Parent(s) & Date Signed

 (If Other Than Parent, Specify Relationship)

 Signature of Investigator & Date Signed

 Signature of Witness & Date Signed

PATIENT IDENTIFICATION

CONSENT TO PARTICIPATE IN A CLINICAL RESEARCH STUDY (Continuation Sheet)

• Adult Patient or • Parent, for Minor Patient

NIH-2514-1 (9-91)

P A 09-25-0099

INFORMED CONSENT FOR RESEARCH PROTOCOL FOR CHLA
THE INSERTION OF THE HUMAN GLUCOCEREBROSIDASE GENE
INTO THE STEM CELLS OF PATIENTS WITH GAUCHER DISEASE

BACKGROUND AND PURPOSE

Gaucher Disease is a genetic disorder caused by the absence of a specific gene from the body. The gene is responsible for producing an enzyme called glucocerebrosidase (GC). Patients with Gaucher Disease do not have this enzyme in their body. The absence of the enzyme causes the body to accumulate certain waste products, called glucocerebrosides, which can damage the body. These waste products can accumulate in many parts of the body, most markedly in the spleen, liver, and bone marrow. The involvement of the spleen often results in an extremely large spleen, which can cause severe problems, such as anemia, bleeding, and a susceptibility to infection. Bone marrow involvement can cause bone pain, bone deformities, and bone fractures. Liver involvement can lead to liver failure. The enlarged spleen and liver may make breathing more difficult. Other organs besides liver, bone marrow and spleen can be affected. The heart can be overworked pumping large amounts of blood to the enlarged organs. The skin can also be affected.

Over the past twenty years, there has been a great deal of research into the treatment of Gaucher Disease. One form of treatment is Bone Marrow Transplantation, using bone marrow from a brother or sister that does not have Gaucher disease. Bone Marrow Transplantation is often successful in reversing or

minimizing the problems associated with Gaucher Disease if an appropriate donor is available. However, the majority of patients will not have a donor. In addition there are substantial health risks associated with a bone marrow transplant. Since a bone marrow transplant is often not possible, alternative treatments have been tested. The best available treatment for Gaucher Disease is known as enzyme replacement therapy. Enzyme replacement therapy with "Ceredase", the commercially available product, is effective and safe in most patients, and you may be receiving this therapy at the present time. However, enzyme replacement therapy must continue for the entire life of a Gaucher disease patient, is very expensive, costing about \$100,000 to \$200,000 per year, and may not be available to many patients with Gaucher disease.

The purpose of this study is to investigate an alternative form of therapy for Gaucher Disease called "gene therapy". We seek to determine if the normal gene responsible for producing glucocerebrosidase can be placed into the blood or bone marrow cells of patients with Gaucher Disease. Certain cells in the bone marrow or blood, called "stem cells," make all of the blood cells in the body, such as red blood cells, white blood cells and platelets. One type of white blood cell made by these stem cells, called a "macrophage," is the cell that builds up the abnormal waste products in Gaucher disease. If we could insert the normal gene for the missing GC enzyme into the stem cells which make macrophages, then possibly these macrophages would no longer build up the toxic waste products, and some of the

problems associated with Gaucher disease could be eliminated.

The placement of the normal gene will be done by removing some bone marrow from the patient with Gaucher Disease, and then treating the bone marrow cells in the laboratory to place the glucocerebrosidase gene into the cells. The treated cells will then be placed back in the patient's body. We will then perform a number of tests to determine whether the new gene is present, and whether or not it is working normally. It is important to realize at this stage that this experimental procedure is only designed to test the safety of these new procedures and that it is not expected that the procedure will result in any direct medical benefit to you at this time.

A number of private companies are supplying materials for the gene transfer part of the study. Genetic Therapy Incorporated (Gaithersburg, MD) is a company that makes the virus that delivers the normal GC gene into cells. Amgen Corporation (Thousand Oaks, CA) and Sandoz Corp (East Hanover, NJ) make growth factors that will be used to treat the bone marrow cells in the laboratory. CellPro Corp. (Seattle WA) makes a product that will be used to purify cells from the bone marrow. Each of these products is under review by the Food and Drug Administration (FDA) to determine if they are safe and effective. Because these four companies are supplying these reagents, they reserve the right to review the records of the treatment. They will maintain your confidentiality, as described on page 8.

POTENTIAL BENEFITS

There are no direct benefits to you from participating in this study. It is possible that the results of the study will lead to a new therapy ("gene therapy") which may benefit you in the future.

STUDY PLAN

The first part of the study plan involves removing a portion of your bone marrow. You will be brought to the operating room and be placed under general anaesthesia. The doctors will then remove about 2 cupfuls of bone marrow from the area on the back of your hip bone known as the posterior iliac crest. The removal is done with needles that are placed through the skin and then through the outer surface of the bone. The removal of bone marrow takes one-two hours and will require an overnight hospital stay.

We will then treat your bone marrow cells in the laboratory for 3-4 days with a virus, which was engineered to carry the normal GC gene into bone marrow cells. Once the GC gene is in the cells, it is permanent, and the cell will pass the GC gene on to all of its daughter cells. The mouse virus has been altered so it cannot grow or spread in your body.

The next part of this experiment will involve putting your treated bone marrow cells back into your body. The cells will be put into your body through an intravenous line. The infusion will take place on the Bone Marrow Transplant Unit and will take four to six hours. You will be monitored closely for side

effects during and after the infusion.

Following this procedure, you will be followed as an outpatient approximately every month for at least one year. We will perform a number of blood tests to determine whether the gene is present in your blood and bone marrow cells, and whether the gene is working normally. The amount of blood drawn will be approximately two-four tablespoons every month. We will request to perform a bone marrow aspiration test from one-to-six times over a two year period. You may refuse to undergo the bone marrow aspirations, if you wish, without being excluded from any other parts of the study. Other X-ray tests and scans may be performed as necessary for your routine clinical care.

Since this study involves a very new approach to patients with Gaucher Disease, it would be important for us to maintain contact with you for at least five years, so that we can monitor your overall progress and determine any possible long term side effects. We request that, if you agree to participate in this study, you do not enter any other gene therapy studies for the next two years, so that we may monitor the results of this study. In the event of your death, we would request an autopsy for the purpose of determining whether the gene has been transferred into important organs, such as the liver or spleen.

It is important to note that participation in this study will in no way alter any of the standard care you may be receiving. If you are currently receiving enzyme replacement therapy, it will continue. There will be no charge to you or your insurance carrier for any of the tests, procedures, or

hospital stays, associated with this study. You will continue to be responsible for the costs of standard care, including enzyme replacement.

ALTERNATIVE TREATMENTS

Participation in this study is completely voluntary and is not intended to be therapeutic. The alternative would be for you not to participate in the study.

POTENTIAL RISKS

The principal risk of the marrow removal is associated with the administration of general anesthesia. General anesthesia can cause problems, such as allergic reactions, breathing or heart problems including the possibility of death. Other risks include bleeding, pain, or infection at the bone marrow aspiration sites. You will receive pain medication to relieve pain if necessary. There is a small possibility that the bone marrow harvest will make you anemic enough to require a blood transfusion.

The laboratory procedures used to treat your bone marrow are experimental, but have been safely used in over 50 patients without any known side effects. There are no known risks in receiving bone marrow cells that contain the new GC gene introduced by the virus. At the time the marrow cells are infused back into your bloodstream, you may experience fever, chills, difficulty breathing, and rarely, a severe allergic reaction that can lead to death. You will be treated for an allergic reaction, if it occurs.

The virus we are using does not cause any known disease in human beings and has been altered so that it cannot replicate itself once it is placed into your body via the bone marrow cells. No side effects have been seen in over 50 patients that have received cells treated with this type of virus, although no patients prior to this study have received cells treated with the GC virus.

One important theoretical risk is the possible development of cancer. It is theoretically possible that the virus that is put into your bone marrow cells could cause the development of cancer in your body. The development of cancer has not been observed in any of the animals or patients treated with this type of virus. It is our opinion that the risk of developing a cancer as a result of this treatment is extremely low.

As in any new form of therapy, there may be risks which are unknown or not anticipated. Because we will only be putting the new GC gene into your bone marrow cells, we do not anticipate that it can be passed on to any children you may have in the future. Participating in this study will not lower the risk of passing Gaucher Disease to your offspring.

PHYSICAL INJURY

The possible risks associated with this study plan have been presented. There is always the possibility that physical injury may occur. The Childrens Hospital Los Angeles maintains professional liability insurance to protect patients from

financial losses resulting from physical injury caused through the fault of the hospital, its employees or its agents. If physical injury occurs in the absence of such fault, the hospital does not provide reimbursement for treatment expenses or other compensation for physical injury suffered as a result of your participation in this study.

If you believe that physical injury has been suffered as a result of participation in this study, you understand that you should contact Dr. Carl Lenarsky or Dr. Donald Kohn at (213) 669-2546. Treatment for such injury will be available under the same financial arrangements as treatment is usually provided.

CONFIDENTIALITY

Case histories, the results of treatment, laboratory and pathologic data, photographs, and x-rays may be published for scientific purposes, but your identity will not be disclosed and confidentiality will be maintained. The Food and Drug Administration (FDA), the Department of Health and Human Services (DHHS), and Genetic Therapy Inc., Amgen, Sandoz, CellPro, and/or their designate(s) may inspect the records relating to your participation in the study. If the study plan or use of the information is to be changed, you will be so informed.

VOLUNTARY PARTICIPATION

This study program has been reviewed, and its consent form has been approved by four boards: 1) the Childrens Hospital Los Angeles Committee on Clinical Investigations (IRB--Institutional

Review Board), 2) the Childrens Hospital Los Angeles Institutional Biosafety Committee, 3) the Recombinant Advisory Committee (RAC) at the NIH, and 4) the Food and Drug Administration (FDA). The approval of these committees does not alter the fact that the final decision to enter yourself in the study program is yours. You understand that you have the right to remove yourself from the study at any time. Dr. Lenarsky or Dr. Kohn will answer any questions you may have regarding this study. Subsequent medical care of yourself at the Childrens Hospital Los Angeles is in no way contingent upon your participation in this study program.

HOW TO OBTAIN INFORMATION

Daytime Monday through Friday, 8 AM to 5 PM, you may call (213) 669-2646. You may leave a message with a secretary, and a doctor will return your call. Evening, nighttime, and weekends or holidays: You may call the hospital number (213) 660-2450, and ask for the on-call doctor for the Bone Marrow Transplant Service. You may be asked to leave a message with the page operator, and the physician on-call will return your call as soon as possible.

If you have any questions regarding your rights as a patient with regard to this study program, you can contact Ms. Gwenn Oki, Administrator of the Committee on Clinical Investigations (IRB) at (213) 669-2265.

SIGNATURE

Your signature below indicates that you have read this document, understand its meaning, have had a chance to ask questions; have these questions answered to your satisfaction; and consent to your participation in this study program.

You hereby give your consent for yourself to be treated according to this clinical study program. You have been given a signed copy of this consent form.

(Patient)

(Physician)

(Witness)

(Date)

(Witness)

(Translator, if applicable)

CURRICULUM VITAE

Name: Stefan Karlsson

Date and Place Birth: May 31, 1950; Akureyri, Iceland

Education:

1970-1976	Cand med et chir (M.D. Degree), University of Iceland Medical Facility
1979-1982	Ph.D. Human Genetics, University College, London

Brief Chronology of Employment:

1977-1979	Physician, Blood Bank, National Hospital, Reykjavik (17 months)
1979-1982	Ph.D. Student-Department of Genetics and Biometry, University College, London
1983-1986	Visiting Fellow and Associate - Clinical Hematology Branch, NHLBI, NIH
1986-1987	Visiting Associate - Developmental and Metabolic Neurology Branch, NINDS, NIH
1988-present	Acting Chief, Molecular and Medical Genetics Section Developmental and Metabolic Neurology Branch, NINDS

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BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME Donald B. Kohn, M.D.	POSITION TITLE Assistant Professor of Pediatrics and Microbiology		
EDUCATION (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)			
INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
University of Illinois, Urbana IL	B.S.	1976	Biology
University of Illinois, Urbana IL	M.S.	1978	Microbiology
University of Wisconsin, Madison, WI	M.D.	1982	Medicine

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HUMAN GENE TRANSFER PROTOCOL

9306-048 Galpin, Jeffrey E., University of Southern California, and Casciato, Dennis A., Shared Medical Research Foundation, Tarzana, California; *A Preliminary Study to Evaluate the Safety and Biologic Effects of Murine Retroviral Vector Encoding HIV-1 Genes [HIV-IT(V)] in Asymptomatic Subjects Infected with HIV-1.*

Date of RAC Approval: 6/7/1993

Date of NIH Approval: 9/3/1993

SCIENTIFIC ABSTRACT OF THE STUDY

This gene therapy protocol involves the use of retroviral vector-mediated gene transfer as treatment for human immunodeficiency virus (HIV)-infected individuals. The protocol employs a genetically engineered, non-replicating, amphotropic murine retroviral vector (N2 IIIBenv) encoding the HIV-1 IIIB envelope (env) protein. Preclinical studies have demonstrated the ability of the N2 IIIBenv vector to induce immune responses in mice, Rhesus monkeys, and baboons. Specifically, the N2 IIIBenv vector was capable of inducing HIV-1 IIIBenv-specific CD8⁺ cytotoxic T lymphocyte (CTL) and antibody responses in these animals. Murine CTL induced by vector transduced cells also exhibited crossreactivity by lysing cells infected with different HIV-1 prototypic strains and clinical isolates. A balanced *in vivo* immune attack by HIV-specific CTL and antibody responses would be expected to eliminate HIV-infected cells and clear cell-free virus, respectively, from an infected individual.

The Phase I placebo-controlled clinical trial involves the direct administration of the N2 IIIBenv retroviral vector or diluent control to HIV-infected, seropositive, asymptomatic individuals not currently receiving anti-retroviral treatment. The direct vector treatment consists of a series of three monthly intramuscular injections of the test article using a two-tier dosing schedule. The packaging cell line, producer cell line, and processed vector material have undergone extensive quality control analysis for the presence of contaminating agents. Treated individuals will be evaluated for acute toxicity and for normal clinical parameters, CD4 levels, HIV-specific CTL responses, and viral load prior to, during, and following treatment. The treated subjects will also be requested to participate in follow-up for at least three years to identify long-term treatment effects and to evaluate their disease progression.

The study protocol is designed to initially evaluate the safety of the direct administration of retroviral vector-mediated gene therapy as a treatment for individuals with a

life-threatening disease. However, functional, biological and clinical disease parameters will be monitored as well to provide a basis for additional human studies.

NON-TECHNICAL ABSTRACT OF THE STUDY

Human Immunodeficiency Virus (HIV) is a virus which has the ability to invade and damage cells of the immune system. It infects and kills that specific group of white blood cells called CD4⁺ T cells. The result of this loss is a serious disabling of the body's immune system, leaving the infected individual open to infections and cancers.

The body also has specific white blood cells known as cytotoxic T lymphocytes (CTL), that function as important killers of cells infected with viruses. HIV-infected individuals who are in an early stage of disease and who are symptom-free could theoretically benefit from a treatment that stimulates these CTL.

Scientists have recently developed a way to insert new genetic information into human cells through use of a disabled virus, called a retroviral vector. The retroviral vector has been designed such that it cannot reproduce to cause disease, but delivers to target cells the genetic codes for the production of certain proteins that resemble important proteins of HIV. The retroviral vector instructs the human target cells to produce such therapeutic proteins. These proteins can then lead to enhanced immune responses targeted at killing HIV-infected cells.

Experiments in mice and non-human primates have shown that treatment with these specific retroviral vectors can stimulate the production of HIV-specific antibodies and CTL without producing any toxic side effects.

This study is designed to determine the safety of the retroviral vector encoding HIV genes for use in humans, and, of secondary importance, whether the vector will have the ability to trigger CTL responses in humans as it does in laboratory animals.

**A PRELIMINARY STUDY TO EVALUATE THE
SAFETY AND BIOLOGIC EFFECTS OF MURINE
RETROVIRAL VECTOR ENCODING HIV-1 GENES
[HIV-IT (V)] IN ASYMPTOMATIC SUBJECTS
INFECTED WITH HIV-1**

HIV-I-02-1292

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OVERVIEW OF THE HIV-1 IIIBenv RETROVIRAL VECTOR CLINICAL PROGRAM

HIV infection is the most rapidly expanding pandemic in recorded time. AIDS slowly and insidiously portends a more certain fatal outcome than even the scourge of medieval plague. Millions are infected today worldwide while tens of millions will contract this illness in the next few years. The rate of transmission is accelerating while the resources to control its spread diminish in many third world countries.

The past teaches us that viral diseases posing threats of this magnitude can be best countered by the host immune system either on its own or with the help of either prophylactic or therapeutic immune stimulation. Roughly 75 separate HIV programs are in progress worldwide and nearly a dozen of these have progressed to clinical trials. However, no product has yet demonstrated clear effectiveness in either preventing or diminishing the advance of this dreaded disease. No product of this type has yet had human clinical approval.

From their fundamental appreciation of HIV/AIDS viral genetics and immunology, most clinician-scientists believe that any effective product will need to not only elicit a specific antibody response, but must also activate a specific cell-mediated immune response. Patients with HIV/AIDS, for example, produce large amounts of antibody to their own illness, and yet most patients have only transient, relative protection from progression of their illness. It is the cellular immune arm of man which protects against malignancy and viruses such as HIV. It is here where the foundation of therapy must be directed and focused.

A unique gene transfer-based approach for the treatment of HIV/AIDS has been developed. The traditionally effective approach represented by attenuated viral vaccines is simulated, but the historical hazard of virulent revertents is avoided. This is accomplished by molecular engineering, which provides genes coding for crucial immunodominant epitopes, yet only about half of the genetic information required to complete a functional HIV virion. The N2 IIIBenv vector programs uninfected antigen-presenting cells of the host to express crucial HIV ENV epitopes in a way that stimulates and/or enhances strong specific antibody and cytotoxic T cell (CTL) responses in mice and non-human primates *in vivo*. It stimulates CTL responses in human peripheral blood mononuclear cells (PBMC) *in vitro*. Critically, CTL reactivated *in vitro* have exhibited cross-reactivity on cells infected with a broad range of different HIV-1 strains and clinical isolates.

The safety of this gene-transfer approach has been confirmed in mice injected with the retroviral vector in comparable routes and doses to the planned clinical trial with no evidence

of toxicity. Studies evaluating the localization and persistence of the vector in mice have also been conducted.

No animal models accurately parallel the clinical or immunological course of human HIV infection and disease. Only human clinical trials can address the efficacy of this new treatment modality.

The initial focus is to establish the human safety of its retroviral vector-mediated gene-transfer treatment. Asymptomatic HIV-1 infected patients will be treated with the genetically engineered, non-replicating, amphotropic murine retroviral vector encoding the HIV-1 IIIB envelope protein (N2 IIIBenv). Three monthly intramuscular injections of the retroviral vector will be administered. Five subjects will receive initial doses of 10^6 cfu and be compared to placebo injections for tolerability and immunogenic activity. Ten additional subjects (five new subjects and the placebo group) will each receive doses at 10^7 cfu. If no significant toxicity is observed in the low dose group, subjects treated will be assessed for acute and chronic toxicity, viral load, antibody and CTL response, and any evidence of clinical efficacy.

Development of highly sensitive assays for the detection of replication-competent retrovirus (RCR) has been part of the exhaustive attention focused on the development of packaging cell lines free of this problem.

The N2 IIIBenv retroviral vector exemplifies a new research direction for treatment of HIV/AIDS. We believe that this clinical protocol for asymptomatic, HIV-1 positive patients has a very favorable risk-benefit ratio. Amplifying both specific antibody and cell-mediated immune response in the HIV infected human host is biologically justifiable, clinically reasonable, and socially desirable. The success of this form of treatment also offers a cost-effective modality which could be administered without economic bias worldwide.

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1.0 OBJECTIVES AND RATIONALE OF THE RESEARCH

The objective of this clinical study is to evaluate the use of retroviral vector-mediated gene transfer for the treatment of human immunodeficiency virus (HIV)-infected individuals. This clinical study will use a genetically engineered, non-replicating murine retroviral vector encoding the HIV-1 IIIB envelope (env) and rev proteins, termed the HIV immunotherapeutic vector [HIV-IT(V)]; and will evaluate the acute toxicity and potential enhancement of immune responses in humans following the direct administration of HIV-IT(V). The intended patient population for this study will be HIV-seropositive, asymptomatic subjects not currently receiving anti-retroviral therapy. It is believed that this patient population will provide relevant safety and immunological data needed to evaluate the use of HIV-IT(V).

The role of immune responses in controlling the disease process of human immunodeficiency virus (HIV) infection is poorly understood. HIV-infected patients exhibit both humoral^{1,2,3,4} and cellular⁵ immune reactivities, and yet continue to show disease progression. Cytotoxic T lymphocyte (CTL) responses mediated by CD8⁺, Class I major histocompatibility complex (MHC)-restricted T cells have been demonstrated in animal models to be a major anti-viral defense mechanism impacting disease severity and duration^{6,7,8}. CTL capable of lysing target cells expressing HIV-1 proteins have been detected in HIV-1 infected patients^{9,10,11,12,13,14,15} and may contribute to controlling the early

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²Robert-Grunoff, M., et al., *Nature*, 316:72-74 (1985).

³Ho, D.D., et al., *N. Engl. J. Med.*, 312:649-650 (1985).

⁴Ho, D.D., et al., *J. Virol.*, 61:2024-2028 (1987).

⁵Popovic, M., et al., *J. Virol.*, 224:497-500 (1984).

⁶Rouse, R.T., et al., *Rev. Infect Dis.*, 10:19-33 (1988).

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¹⁵Koenig, S., et al., *J. Immunol.*, 145:127-135 (1990).

stages of HIV-1 infection¹⁶. It is apparent, however, that immune responses resulting from natural HIV infection are ultimately inadequate for stabilization or recovery, perhaps because of suboptimal viral antigen presentation (e.g., down-regulation of MHC)¹⁷ by virus-infected cells. More potent HIV-specific CTL activity may have a significant impact on halting HIV disease progression because CTL are capable of eliminating virus-infected cells, the reservoir of replicating HIV.

Conventional product strategies (inactivated virus, soluble proteins) characteristically elicit humoral antibody responses, whereas live attenuated viral products appear to induce both potent antibody and cellular immune responses. The effectiveness of attenuated viral products is likely attributable in part to their ability to provide foreign viral proteins to the intracellular antigen processing pathways for effective CTL activation. However, the use of live attenuated viral products presents significant safety concerns, particularly regarding HIV. Therefore, alternative approaches, such as the use of HIV-IT (V), may provide for more consistent CTL activation and is important to test these effects in combating HIV disease progression.

Viral infection and intracellular synthesis of viral proteins are usually required for *in vivo* priming of CD8⁺, Class I MHC-restricted CTL^{18,19,20,21}. In contrast, inactivated virus and soluble proteins do not consistently induce CTL *in vivo*²². Endogenously synthesized foreign proteins in virus-infected cells are degraded within the cytoplasm into protein fragments that associate with Class I MHC. This antigen-MHC complex, when transported to the cell surface, can lead to activation of CD8⁺ CTL.

Retroviral vectors are very efficient at introducing genes into host cells, and thus, represent an effective means of providing foreign antigens to the intracellular antigen

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¹⁹Braciale, T.J., et al., *Immunol. Rev.*, 98:95-114 (1987).

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²¹Townsend, A., et al., *Nature*, 334:443-448 (1989).

²²Bevan, M.J. *Nature*, 342:478-479 (1989).

presentation pathway. Because retroviral vectors are non-replicating and non-cytopathic to cells, one would predict negligible perturbation of normal cellular processes following retroviral vector transduction. Therefore, retroviral vector-mediated gene transfer may provide advantages for antigen presentation compared to that of replicating vector systems, or the natural viral infection. HIV-IT (V) is capable of delivering the genes that encode the HIV-1 env/rev proteins. These proteins appear in the cytoplasm of HIV-IT (V) transduced cells as endogenous proteins, capable of entering the endogenous antigen presentation pathway necessary for CD8⁺ CTL induction. Moreover, HIV-IT (V)-delivered env gene product is also expressed on the cell surface and can enter the exogenous pathway, thus activating antibody responses. The preclinical studies described here demonstrate the ability of non-replicating murine retroviral vectors to induce HIV-specific humoral and cellular immune responses, particularly CTL responses, in murine and non-human primate models.

The potential benefits of using HIV-IT(V) to induce HIV-specific immune responses in HIV-infected patients are:

- HIV antigens produced intracellularly should be processed and presented to the immune system in the context of MHC-I,
- HIV antigens should be presented by treated cells in the absence of potentially perturbing HIV infection processes, and
- augmented HIV-specific CTL and antibody responses should be induced.

The current treatment for HIV infection involves the use of reverse transcriptase (RT) inhibitors, e.g., AZT, ddI, DDC, or D4T as single or combination therapy to slow viral replication. Triple therapy using unique RT inhibitors along with either immune modulators or selectively different HIV-1 inhibitors also demonstrates promise. These therapies show partial clinical efficacy over relatively short periods of time, but do not appear to effectively halt disease progression. In addition, elimination of previously infected cells is not proven to occur with these interventions. Moreover, HIV is capable of drug resistant

mutation^{23, 24} following prolonged treatment with AZT or any single RT inhibitor. It is unclear whether combination therapy will be totally successful in suppressing this emergence. For these reasons, alternative therapies to be used singly or in combination are actively being sought.

Due to this lack of any proven long-term anti-viral therapy for HIV-1 infected patients and the predictable terminal outcome of this infection, (i.e., death) in over 75% of infected individuals, the potential benefit of gene therapy using retroviral vectors far outweighs the perceived risks associated with this technology.

The induction of CD8⁺ cytotoxic T lymphocytes (CTL) potentially reduces the lymphocyte and monocyte-macrophage reservoirs of HIV-1. Reduction in infected lymphocyte and macrophage populations may prevent cell or tissue injury such as progressive neurotoxicity or progressive immune deterioration from viral direct, indirect, or induced toxins. Therefore, successful treatment-induced activity (CTL and antibody) may halt disease progression in asymptomatic subjects and reverse disease manifestations in more advanced HIV-infected individuals provided their immune system is capable of generating appropriate immune responses.

2.0 VECTOR CONSTRUCT, PACKAGING AND PRODUCER CELL LINES

2.1 VECTOR CONSTRUCT

The N2 IIIBenv provector backbone is a modified form of the N2 retroviral provector²⁵ with the gag AUG mutated to ATT. The insert was obtained from pAFenv SV₂neo²⁶ and consists of the env and rev genes from HIV-1 IIIB as the 5' genes driven from the MoMLV LTR. In addition, the neo^r gene driven from the SV40 early promoter is included as the 3' gene to allow selection of transduced cells. The construction and component parts of the N2 IIIBenv provector are shown in Figure 1.

²³Gao, Q., et al., *Antimicrobial Agents and Chemotherapy*, 37:130-133 (1993).

²⁴Mohri, H., et al., *Proc. Natl. Acad. Sci. USA*, 90:25-29 (1993).

²⁵Aramentano, et al., *J. Virol*, 61:1647-1650, (1987).

²⁶Warner, J.F., et al., *AIDS Research and Human Retroviruses*, 7:645-655 (1991).

2.1.1 Sequence Analysis of the Vector

A scale diagram and functional description of the components of the N2 IIIBenv provector plasmid are included in Appendix A.

Also provided in Appendix A is the complete report from Lark Technologies including a nucleotide sequence analysis, a restriction enzyme analysis and the amino-acid translation of the N2 IIIBenv provector. One nucleotide difference was observed between the HIV-1 IIIBenv sequences from that expected. This change does not change the amino acid sequence.

2.2 PACKAGING CELL LINE

A novel amphotropic packaging cell line (designated DA) was created from a parental canine cell line (D-17, ATCC #CCL183) by sequentially transfecting the genes for the carrier structural proteins, gag/pol and env^{am} (amphotropic envelope) into the parent cell line. The structural gene, gag/pol, was derived from the Moloney murine leukemia retrovirus²⁷ (MoMLV) whereas the envelope gene, env^{am}, was derived from the murine leukemia retrovirus MLV 4070A²⁸. The packaging cell line continuously expresses the corresponding MLV structural proteins and as a result, produces "empty" virion particles²⁹. When a retroviral provector, such as N2 IIIBenv, is introduced into the packaging cell line, the MLV structural proteins package the retroviral vector genome into the virion particle and nascent retroviral vector accumulates in the cell medium.

2.2.1 Parent Cell Line

The canine cell line D-17 was originally chosen as the parent for the packaging cell line because its genome was expected to lack homology to murine C-type retroviruses. This was confirmed by Southern blot analysis which showed no specific cross hybridization between D-17 genomic DNA and the MLV gag/pol and env^{am} used in the construction of the packaging cell line. The parent cell line has also been

²⁷Miller, et al., *Mol. Cell. Biol.* 5:431-443, (1985).

²⁸Chattopadhyay, et al., *J. Virol.*, 39 :777-791, (1981).

²⁹Cornetta, et al., *Human Gene Therapy*, 2:5-14, (1991).

treated with IUdR and dexamethasone and tested negative for the presence of cryptic retroviruses.

2.2.2 Construction of Packaging Cell Line

The D-17 canine cell line was initially transfected with the plasmids pSCV10 and pFR400, which express MLV gag/pol and a methotrexate resistance marker, respectively, and an individual subclone selected which expressed high levels of MLV gag proteins. This clone was then transfected with pCMV env^{am} Dra and pUT507³⁰. The pCMV env^{am} Dra plasmid encodes the MLV amphotrophic envelope gene (env^{am}) and the pUT507 plasmid provides a phleomycin dominant selectable resistance marker (phleo^r).

After selection in phleomycin, individual colonies were isolated, expanded and tested for production of MLV gag and env^{am} by Western blot analysis. A resulting packaging line clone, 4(15)SC1 #23, designated DA, was selected.

The plasmids encoding the structural genes, pSCV10 and pCMV env^{am} Dra, were constructed at Viagene, Inc. These plasmid constructs use the human cytomegalovirus (CMV) early transcriptional promoter³¹ for structural gene expression. The MoMLV gag/pol was obtained from the MoMLV proviral plasmid, MLV-K³². The env^{am} gene was obtained from the murine retrovirus MLV 4070A proviral clone³³. The gag/pol and env^{am} constructs use the SV40 late transcriptional termination signal³⁴.

2.3 GENERATION OF THE PRODUCER CELL LINE (DA/N2 IIIBENV) USED TO GENERATE HIV-IT (V)

The N2 IIIBenv retroviral vector, designated HIV-IT (V), is produced by introducing the N2 IIIBenv provector DNA into an amphotropic packaging cell line. This results in

³⁰ Mulsant, et al., *Somat. Cell. Mol. Genet.*, 14:243-252, (1988).

³¹ Boshart, et al., *Cell*, 41:521-530, (1985).

³² Miller, et al., *Mol. Cell. Biol.*, 5:431-443, (1985).

³³ Chattopadhyay, et al., *J. Virol.*, 39:777-791, (1981).

³⁴ Southern and Berg, *J. Mol. App. Genetics*, 2:327-341 (1982).

the production of full length and spliced RNAs (vector genomes), which are encapsidated into non-replicating vector particles. When transduced into target cells, the full length provector has the capacity to produce both HIV-1 IIIB env and rev proteins, whereas the spliced vector can produce only rev.

Rather than directly transfecting the vector into the packaging cell line, the N2 IIIBenv vector was transduced into the canine packaging cell line (DA). This procedure was favored, as transduction generates a defined proviral integration event and leads to higher titer producer cell lines than those generated by transfection³⁵.

Amphotropic PCLs are blocked for transduction by amphotropic vector as the cell receptor is occupied (a process known as "viral interference"). To overcome this transduction blockage, vectors containing alternative envelopes were first generated. One such alternative is the VSV G-protein, which binds to cell receptors other than the amphotropic receptor. The VSV G-protein is incorporated into the surface of a retroviral vector in a process termed "pseudotyping". VSV G-pseudotyped retroviral particles can efficiently transduce a wide array of cell lines³⁶.

A human embryonal kidney cell line 293³⁷ containing MoMLV gag/pol (termed 293 2-3) was cotransfected with N2 IIIBenv provector and the plasmid, MLP-G, which expresses the VSV-G envelope protein (constructed at Viagene). The VSV-G pseudotyped N2 IIIBenv retroviral particles were used to transduce the canine packaging cell line (DA), and individual producer cell clones resistant to the antibiotic, geneticin (G418), were isolated.

The clones were screened for maximal N2 IIIBenv vector production as determined both by their neo^r titer and their ability to transduce and express HIV-1 IIIBenv in a human fibrosarcoma cell line, (HT-1080), as assessed by Western blot analysis. An

³⁵Miller, et al., *Somat. Cell Mol. Genet.*, 12:175-183, (1986).

³⁶Emi, et al., *J. Virol.*, 65:1202-1207 (1991).

³⁷ATCC # CRL 1573.

individual producer cell clone, DA/N2 IIIBenv.2C3, was selected using the above criteria.

2.3.1 Characterization of DA/N2 IIIBenv.2C3 Producer Cell Line (PCL)

(1) Numbers of Transduced Copies Per Cell:

Southern blot analyses of DA/N2 IIIBenv.2C3 DNA probed with neo^r (obtained from N2 IIIBenv) indicate the presence of a single full length proviral copy per cell. An additional band consistent with integration of a rev proviral copy was also observed.

(2) Expression of HIV-1 IIIBenv/rev:

The N2 IIIBenv retroviral vector construct necessarily encodes the genes for both HIV-1 IIIBenv and rev as rev is required for env expression. Because HIV-1 IIIBrev is encoded by two separate exons, a splicing event is required for the generation of rev mRNA. As shown in Figure 2, when N2 IIIBenv is introduced into a retroviral packaging cell, spliced and unspliced RNA species are produced. Because both spliced and unspliced RNA's contain the packaging signal, both species can be packaged within the PCL to produce either HIV-1 IIIBenv/rev virions or HIV-1 IIIBrev virions. The full length RNA will deliver and express both HIV-1 IIIBenv and rev proteins, whereas the spliced rev RNA has the capacity to express only HIV-1 IIIBrev. In order to assess the relative frequency with which the rev and env/rev vectors would be expected to be delivered *in vivo*, mouse BC10ME cells were transduced at multiplicity of infection (MOI) of 1 and 10 in tissue culture. Southern blots of DNA from pooled transductants showed that the two provectors were present at a ratio of approximately 1:1.

To examine HIV-1 env protein production in transduced cells, increasing amounts of HIV-IT (V) were used to transduce human HT-1080 cells. Forty-eight hours after transduction, cell lysates were prepared and analyzed by Western blotting using an anti-HIV-1 env-specific monoclonal antibody (Figure 3). Both full

length and processed gp160 and gp120 HIV-1 env proteins are expressed in the transduced cells. Densitometric analysis of the autoradiograph was used to determine the amount of HIV-1 env protein expressed per cell (Figure 3). The level of HIV-1 env expression correlated with multiplicity of infection of HIV-IT (V). Human, chimpanzee, Rhesus monkey, and mouse primary and transformed cell lines can be transduced with the N2 IIIBenv vector. Figure 4 shows expression of HIV-1 gp160 and gp120 in the N2 IIIBenv transduced cells.

2.3.2 Growth Conditions of the Producer Cell Line and Purification of HIV-IT (V)

HIV-IT(V) is prepared from the DA/N2 IIIBenv.2C3 producer cell line grown in a bioreactor. The producer cell line is grown in media consisting of DMEM (Irvine Scientific) supplemented with glucose, 1X non-essential amino-acids, 10mM Hepes and 10% FBS (gamma irradiated, Hyclone) in a 5L bioreactor (New Brunswick Scientific) on microcarrier beads. Currently, cell densities of 1.5×10^6 /ml are routinely attained, yielding unprocessed vector titers (neo^r colony forming units, cfu) of approximately 5×10^6 cfu/ml.

The vector preparation is removed from the bioreactor, and purified by a proprietary process. The vector is formulated and stored frozen at -80°C . After purification, HIV-IT(V) titers of greater than 1×10^7 cfu/ml are usually attained.

2.3.3 Safety Testing of Producer Cell Line

The DA/N2 IIIBenv.2C3 producer cell line has been characterized and subjected to numerous quality control analyses. The testing is described in Figure 5. This cell line is the subject of a master file submitted to the FDA.

2.3.4 Safety Testing of the Vector, HIV-IT (V)

In addition to comprehensive testing of the producer cell line, HIV-IT (V) has also been extensively tested to ensure safety and biological activity. Tests for

detection of replication-competent retroviruses (RCR) are described in more detail in Section 2.3.5(d), below.

2.3.5 Safety Features of the Producer Cell Line

Risks of gene transfer using retroviral vectors have been comprehensively addressed by others in animal models and most recently in human subjects³⁸. As discussed below, a significant effort in the selection and development of the HIV-IT(V) has been made to minimize the potential risks associated with this technology.

(a) Canine Cells as Producer Lines:

To increase the safety profile of producer cell lines, Viagene's PCL has been generated from a canine cell line. Southern hybridization analysis has been performed which shows that parental D-17 cells lack genomic sequences homologous to MoMLV. In addition, no reports have been found of authentic endogenous canine retroviruses. Thus, the PCL background genome contains no known sequences homologous with the vector or the vector particle structural genes.

Murine cell lines have been widely used to develop PCLs for use in gene transfer. It is possible to generate RCR from the very large number of homologous, defective, endogenous proviruses found in murine cells^{39,40,41}. In addition, murine cell lines can produce endogenously encoded xenotropic retrovirus either spontaneously, or after induction^{42,43,44}. Furthermore, many defective endogenous proviruses (retrovirus-like genomes such as VL30 sequences) in the murine genome are expressed and the RNA can be recognized by the retroviral structural gene products of murine PCLs. These RNA genomes can be incorporated into retroviral vectors, and can be delivered to

³⁸ Cornetta, et al., *Human Gene Therapy*, 2:5-14, (1991).

³⁹ Steffen and Weinberg, *Cell*, 15:1003-1010, (1978).

⁴⁰ Canaani and Aaronson, *Proc. Natl. Acad. Sci., U.S.A.*, 76:1677-1681, (1979).

⁴¹ Stoye and Coffin, *J. Virol.*, 61:2659-2669, (1987).

⁴² Aaronson and Dunn, *J. Virol.*, 13:181-185, (1974).

⁴³ Stephenson and Aaronson, *Proc. Natl. Acad. Sci., U.S.A.*, 71:4925-4929, (1974).

⁴⁴ Aaronson and Stephenson, *Biochim. Biophys. Acta.*, 458:323-354, (1976).

target cells with an efficiency at least comparable to that of the desired vector^{45,46}. These observations emphasize that canine cell lines possess advantages for the production of murine retroviral vectors for human gene transfer therapies.

(b) Split Structural Genes for gag/pol and env^{am}:

One of the most important safety concerns arises from the potential propensity of PCLs to generate RCR after introduction of a retroviral vector. This can occur as a result of homologous recombination between the provector DNA and the DNA encoding the MLV structural genes (gag/pol and env^{am}) present in the PCL⁴⁷. This potential problem can be ameliorated by generating a PCL which contains genetically unlinked MLV structural genes (split genome). The split genome strategy was used in the construction of the canine packaging cell line, DA. However, it should be noted that the use of a split genome can not guarantee that recombination will not take place.

(c) Reduced Sequence Homology Between Murine Retroviral Elements in the Producer Cell Line:

There is reduced sequence homology between the MLV structural genes and N2 IIIBenv retroviral DNA because the structural genes in the producer cell line use heterologous transcriptional promoter and termination signals instead of MoMLV LTRs. Multiple homologous recombination events within minimal sequence overlap would be required to generate a RCR (Figure 6).

Approximately 700 base pairs of homologous sequence exist between the gag regions of the N2 IIIBenv vector and pSCV10 (the plasmid used to express MoMLV gag/pol in the PCL). However, only recombinational events upstream from the ATG->ATT mutation in N2 IIIBenv would result in RCR because a downstream recombinant could not make gag/pol protein. The functional overlap for homologous recombination is therefore less than 60 base pairs. 550 base pairs of apparent overlap

⁴⁵ Scolnick, et al., *J. Virol.*, 29:964-972, (1979).

⁴⁶ Scadden, et al., *J. Virol.*, 64:424-427, (1990).

⁴⁷ Miller, *Human Gene Therapy*, 1:5-14, (1990).

exist between pSCV10 and pCMVenv^{am} Dra (the plasmid used to express amphotropic envelope in the PCL), but these plasmids are derived from two different murine leukemia viruses, MoMLV and MLV 4070A, respectively, which differ in their nucleotide sequences. This design makes the probability of generating RCR low.

(d) Testing for Replication-Competent Retroviruses

A number of novel reagents and procedures have been used to detect RCR in conjunction with a commercially available validated extended S⁺L⁻ test. The tests used are summarized below.

Test	Measurement
Extended S ⁺ L ⁻	Focus formation
<i>Mus dunni</i> marker rescue (MdH)	Hygromycin resistance (hygro ^r)
<i>Mus dunni</i> marker rescue (MdC)	β-gal gene expression
<i>Mus dunni</i> cocultivation with PCL	Marker rescue or S ⁺ L ⁻ on the cell supernatant.

The marker rescue assays test vector preparations using a *Mus dunni* cell line that has been transduced with a retroviral vector encoding either hygro^r (MdH) or beta-galactosidase (MdC). This cell line was chosen as it is readily infected by amphi-, eco- (except MoMLV), xeno- and poly-tropic retroviruses and is very permissive for propagation of RCR. When MdH cells are infected with a RCR, the virus "rescues", by transcomplementation, the hygro^r retrovirus which can then transduce a target cell. If the target cells are selected with hygromycin, formation of resistant colonies indicates the presence of RCR. The MdC assay is similar in principle, however, a β-gal containing vector is rescued and assayed on target cells using X-gal staining. All HIV-IT (V) preparations tested from the DA/N2 IIIBenv.2C3 PCL have been negative using these assays. In our experience, these assays are roughly comparable in sensitivity. The extended S⁺L⁻ assay which is commercially available, is at least as sensitive as other assays.

Mus dunni cocultivations are performed to amplify any low level RCR in a test cell line. Producer cell lines are cocultivated with *Mus dunni* cells for 15 days, and the resulting supernatants are tested using MdC, MdH, or extended S⁺L⁻ assays.

The producer cell line DA/N2 IIIBenv.2C3 was tested by *Mus dunni* cocultivation for the generation of RCR (Figure 7). Positive controls are the DA/N2 IIIBenv.2C3 cells cocultivated with *Mus dunni* cells and spiked with increasing numbers of Md/MA cells (*Mus dunni* cells infected with the hybrid MoMLV-4070A amphotropic virus MA). These data show no detection of RCR in the producer cells. The controls indicate that approximately one cell infected with RCR can be detected in this test system. We have performed similar testing on the producer cell line before banking, on the banked cell line, and on cells post production. These have given uniformly negative results.

3.0 PRECLINICAL STUDIES

Preclinical studies have utilized three animal models to test the immunobiological activity of HIV-IT (V). The study models were normal mice, Rhesus monkeys, and baboons. In addition, *in vitro* immune stimulation studies have been conducted with lymphocytes obtained from HIV-1-infected individuals. Animal studies have examined and demonstrated the ability of HIV-IT (V) to stimulate HIV-1 envelope-specific CTL and antibody responses *in vivo* in the animal models. No toxicological effects have been observed in animals receiving HIV-IT (V) treatment.

These preclinical studies involving direct administration of HIV-IT (V) provided the foundation for the planning of clinical trials in humans. The study parameters were: dose, number of injections, types of induced immune responses, and the establishment of human CTL monitoring systems.

3.1 MOUSE MODEL

3.1.1 CTL Induction By HIV-IT (V)

The following murine studies employed the BALB/c syngeneic cell line BC10ME (BC) and the HIV-IT (V)-transduced BC10ME cell line (BCenv) as control and HIV-1 env-expressing CTL targets, respectively. BCenv transduced cells express the HIV-1 envelope and rev (env/rev) proteins, and the neo^r selectable marker protein. Mice were immunized with one or two injections of HIV-IT (V), and the primed splenocytes were stimulated *in vitro* with irradiated BCenv cells. The *in vitro* stimulation is routinely used to increase the number of cytotoxic cells for optimal detection in the CTL assay. The resulting effector cells were tested for CTL activity by measuring lysis of radiolabeled BCenv and control BC target cells in a 4 - 5 hour chromium (⁵¹Cr)-release assay^{48,49,50}.

Examination of the accumulated data in the mouse model reveals variability in the ability to generate CTL responses to HIV-IT (V) (Figure 8). These data reveal no statistically significant differences in CTL induction with one or two injections, or over the range of vector doses administered (10^4 - 10^7 cfu). However, for a number of reasons, it is not surprising that considerable variability in CTL induction was observed in these experiments. Our experimental approach throughout these studies was to examine parameters which might impact CTL induction. Firstly, these data include CTL induction by fourteen different HIV-IT (V) lots and five different HIV-IT (V) formulations. Secondly, Figure 8 includes experiments designed to optimize CTL responses, examining such parameters as the route of injection, the number of administrations, the injection schedule and the volume of HIV-IT (V) injected. Finally, the induction of a specific CTL response is a result of interactions in a complex biological system where some variation in response is expected.

⁴⁸Warner, J.F., et al., *AIDS Research & Human Retroviruses*, 7:645-655 (1991).

⁴⁹Warner, J.F., et al., *J. Immunol.*, 122:575-581 (1979).

⁵⁰Brunner, K.T., et al., *Immunology*, 14:181 (1968).

3.1.1.2 Dose Response

To more specifically assess the dose range required to induce CTL activity using a single lot of crude, formulated HIV-IT (V), mice were given a single IP injection of various doses of HIV-IT (V). CTL induction was achieved with a single dose of 1.6×10^6 HIV-IT (V) cfu, whereas injection of 8×10^5 cfu or less did not induce cytotoxic activity (Figure 9A). However, two HIV-IT (V) injections of 9×10^4 cfu or greater resulted in marked cytotoxic activity (Figure 9B). Thus, cytotoxic activity could be obtained following single or multiple injections of HIV-IT (V) in a dose-dependent manner. These studies demonstrated that administration of multiple low-dose treatments or a single high-dose treatment can induce comparable CTL responses.

3.1.1.3 HIV-IT (V) Encoded Proteins Induce the CTL Response

The specificity of the effector CTL induced with HIV-IT (V) was examined. As described previously, the N2 IIIBenv provector used to produce HIV-IT (V) encodes three gene products: the HIV-1 env protein; the HIV-1 rev protein; and the neomycin resistance (neo^r) protein. Theoretically, the HIV-IT (V)-induced CTL could be directed against each of these provector-encoded proteins or vector associated components. Therefore, studies were designed to evaluate the specificity of the HIV-IT (V)-induced CTL response.

HIV-IT (V)-induced CTL effectors were tested for their ability to lyse vector-transduced target cells expressing β -galactosidase (β -gal) and neo^r , but not expressing HIV-1 env or rev proteins (BC- β gal), and such lysis compared to the BCenv target cell which expresses HIV env, rev and neo^r proteins. BALB/c mice were injected IP once with purified formulated HIV-IT (V) (4×10^5 cfu) and the primed splenocytes were stimulated *in vitro* with irradiated BCenv cells. The resulting HIV-IT (V) induced CTL exhibited lysis of only the HIV-1 env/rev/ neo^r expressing BCenv target, whereas negligible background lysis was observed on the

BC- β gal (β gal/ neo^r) targets (Figure 10). Furthermore, multiple injections (up to three) of HIV-IT (V) did not induce effectors capable of causing any BC- β gal lysis. These results demonstrated that the CTL induced by HIV-IT (V) were specific for HIV-1 env/rev determinants, and that the CTL activity was not directed against the neo^r gene product.

3.1.1.4 Phenotype of Cytotoxic Effector Cells

In additional experiments, an attempt was made to determine the phenotype of the HIV-IT (V)-induced cytotoxic effector cell population by depletion of subpopulations of T cells (i.e., CD4⁺ and CD8⁺) using specific mouse monoclonal antibodies (MAb) plus rabbit complement. Preliminary data indicate that the CD8⁺ T cell subpopulation was responsible for the measured cytotoxic activity since target cell lysis was abrogated following CD8⁺ T cell depletion, but not following CD4⁺ T cell depletion compared to control treatments. The existing data indicate that the majority of cytotoxic effector cells induced by injection of mice with HIV-IT (V) are CD8⁺, CD4⁻ cytotoxic T lymphocytes.

Conventional CD8⁺ CTL are known to recognize epitopes of viral antigens in association with self class I MHC molecules⁵¹. We have preliminary evidence that direct vector induced CTL are class I MHC restricted to the murine H-2D^d molecule. This is in agreement with HIV-1 IIIBenv-specific CTL responses generated in the BALB/c mouse using N2 IIIBenv transduced syngeneic fibroblasts⁵² and with a recombinant vaccinia vector⁵³. Therefore, these HIV-IT (V)-induced CTL appear to be class I MHC restricted and the effector cell is CD8⁺.

3.1.1.5 HIV-1 env/rev Specificity of CTL

⁵¹Zinkernagel, R.M., et al., *Adv. Immunol.*, 27:51-178 (1979).

⁵²Warner, et al., J.P., et al., *AIDS Research and Human Retroviruses*, 7:644-655 (1991).

⁵³Takahashi, H., et al., *Science*, 246:118-121 (1989).

Further analysis of the HIV-1 env specificity of the HIV-IT (V)-induced CTL responses was performed using synthetic peptides derived from the hypervariable V3 domain of HIV-1 IIIBenv, gp120 (i.e., amino acids 307-330). This sequence was previously shown to provide a dominant epitope recognized by HIV-1 neutralizing antibodies^{54,55,56,57} as well as murine CTL^{53,58}. CTL induced by IP injection of 10^6 cfu of crude HIV-IT (V) were examined for their ability to recognize and lyse BC cells coated with the P18IIIB peptide (RIQRGPGRAFVTIGK) or the P18MN peptide (RIHIGPGRAFYTTKN) derived from the hypervariable V3 loop region of the envelope protein of the HIV-1 IIIB and HIV-1 MN strains, respectively⁵³. The CTL showed lysis of the P18IIIB peptide-coated (5 μ M) BC cells at approximately 60% of that observed on BCenv target cells expressing the entire env and rev proteins (Figure 11). Moreover, crossreactivity was observed using target cells coated with the P18MN peptide (5 μ M) derived from the equivalent V3 region of the HIV-1 MN isolate. Thus, within the population of cytotoxic effector cells induced by injection of HIV-IT (V), there exist CTL that recognize determinants within the V3 loop region of gp120 amino acid residues 315-329 derived from the HIV-1 IIIB isolate. These CTL also appear to recognize and cross-react with a peptide derived from the equivalent region of the HIV-1 MN isolate.

Further studies have evaluated the CTL recognition of determinants outside the immunodominant V3 loop region of the HIV-1 IIIBenv protein. A retroviral provector construct was designed to express a V3-region-deleted, HIV-1 IIIBenv protein, in which the gene sequence coding for amino acids 303-338 was removed (Δ V3). BC cells were transduced with this Δ V3 provector construct (BCenv Δ V3)

⁵⁴Rusche, J.R., et al., *Proc. Natl. Acad. Sci. (USA)*, 85:3198-3202 (1988).

⁵⁵Javaherian, K., et al., *Proc. Natl. Acad. Sci. (USA)*, 86:6768-6772 (1989).

⁵⁶Palker, T.J., et al., *Proc. Natl. Acad. Sci. (USA)*, 85:1932-1936 (1988).

⁵⁷Goudsmit, J., et al., *Proc. Natl. Acad. Sci. (USA)*, 85:4478-4482 (1988).

⁵⁸Takahashi, H., et al., *Proc. Natl. Acad. Sci. (USA)*, 85:3105-3109 (1988).

and expressed the modified HIV-1 env protein. Subsequent studies have shown that BCenvΔV3 target cells did not express V3 loop region, but were still subject to lysis by HIV-1 env/rev-specific CTL.⁵⁹

BALB/c mice were injected with crude HIV-IT (V) as above, and splenocytes stimulated multiple times *in vitro* with irradiated BCenv and subsequently with BCenvΔV3. The effector cells were tested on BCenv, BCenvΔV3, and BC control target cells. The HIV-IT (V)-induced CTL exhibited substantial lysis of BCenv and BCenvΔV3 target cells compared to BC control cells (Figure 12). Furthermore, these CTL were previously shown to recognize the P18IIIB and P18MN peptides. Therefore, this CTL population contains effector cells that recognize not only determinants contained within the V3 hypervariable region, but also HIV-1 env epitopes outside the V3 hypervariable region that are potentially more conserved epitopes. The CTL epitopes recognized outside the V3 hypervariable region could be determinants located in the gp120 or gp41 portions of the env protein or determinants residing in the rev protein. Envelope-specific lysis by HIV-IT (V) induced CTL was confirmed by testing BC cells infected with a vaccinia-gp160 recombinant virus, which expressed the HIV-1 env, but not the rev or neo^r genes.

3.1.1.6 Ability of Induced CTL to Lyse Cells Infected with Divergent HIV-1

Strains

An effective anti-HIV-1 treatment must be designed to generate responses that are capable of recognizing the multiple virus isolates or strains of HIV-1 present in patients. Extensive serological analysis of neutralizing antibodies and DNA sequence analysis of the V3 hypervariable region (principal neutralizing determinant) of the HIV-1 envelope proteins of different HIV-1 isolates has indicated that most HIV-1 specific neutralizing antibodies are virus strain-specific,

⁵⁹Chada, et al., *J. Virol*, In Press.

i.e., they neutralize only the homologous HIV-1 viral strain⁶⁰. This serological approach to virus typing has suggested the existence of several major classes of HIV-1 strains in North America and further major divergence worldwide⁶⁰. However, CTL crossreactivity on the various HIV-1 prototypic strains and clinical isolates has not been extensively evaluated. Therefore, the demonstration of CTL crossreactivity on cells infected with different HIV-1 isolates may indicate the importance of CTL immune responses in treating patients infected with diverse clinical HIV-1 strains.

Ideally, CTL activity should be evaluated on HIV-1 infected target cells. However, mouse cells do not have the appropriate cell surface receptor (i.e., CD4) for HIV-1 infection, and HIV-1 infected human cells do not possess the appropriate MHC restriction elements necessary for mouse CTL recognition. Therefore, an *in vitro* target cell system combining the elements of susceptibility to HIV-1 infection and a mouse MHC molecule has been established to facilitate the analysis of CTL activity on targets infected with different HIV strains.

The Hela-T4 cervical carcinoma cell line⁶¹ can be productively infected with different HIV-1 isolates, as demonstrated by p24 antigen detection, syncytium formation, and cytopathic effects. This cell line has been engineered to express the mouse H-2D^d MHC molecule and is called Hu/D^d. The Hu/D^d cell line expresses the appropriate MHC restriction element (i.e. H-2D^d) required for mouse CTL recognition. Using this target cell system, HIV-1 env-specific mouse CTL should be able to recognize and lyse HIV-1 infected human Hu/D^d cells because the target cells can present HIV-1 antigens in the context of the mouse class I MHC molecule. This genetically engineered target cell system can facilitate the *in vitro* CTL

⁶⁰LaRosa, G.J., et al., *Science*, 249:932-935 (1990).

⁶¹Madden, et al., *Cell*, 47:333-348 (1986).

analysis of potential clinical cross-coverage of cells infected with different HIV-1 strains.

In previous studies, the HIV-IT (V) transduced syngeneic fibroblast cell line (BCenv) has been used to induce HIV-1IIIB env-specific CTL in BALB/c mice. CTL crossreactivity was then evaluated on Hu/D^d target cells infected with different HIV-1 isolates. BCenv-induced CTL were analyzed for their ability to lyse target cells infected with different HIV-1 prototypic strains as well as HIV-1 clinical isolates obtained from HIV-1-infected patients. These CTL were able to lyse Hu/D^d target cells infected with the HIV-1 IIIB, HIV-1 MN, HIV-1 SF2, HIV-1 WMJII, and HIV-1 CC prototypic viral isolates, but not uninfected Hu/D^d control targets (Figure 13)⁶². Interestingly, the Hu/D^d cells infected with HIV-2 were not lysed suggesting that HIV-2 infected cells do not express CTL epitopes shared with HIV-1 isolates. Furthermore, BCenv-induced CTL were capable of lysing Hu/D^d target cells infected with several clinical isolates of HIV-1 obtained from HIV-infected patients (Figure 14A), as well as sequential HIV-1 clinical isolates that have become resistant to AZT (Figure 14B).

The results show that CTL induced with HIV-IT (V)-transduced cells have a broad reactivity against a number of HIV-1 prototypic strains and clinical isolates. These results also suggest that the range of CTL reactivity is less restricted than that seen for type-specific antibody responses perhaps due to recognition of conserved epitopes by CTL effectors. Therefore, the Hu/D^d target cell system has demonstrated that CTL induced by cells transduced with a retroviral vector encoding HIV-1 IIIBenv, are capable of recognizing diverse strains of HIV-1.

3.1.2 Antibody Induction

The antibody response, specific for the HIV-1 env protein (i.e., gp160/120) has been examined in mice. Following administration of HIV-IT (V). Shown in Figure 15

⁶²Chada, et al., *J. Virol.*, (1993) In Press.

are the results from an experiment in which BALB/c mice were injected IM three times with 1×10^5 cfu crude HIV-IT (V). These mice were bled 9-15 days post injection, and sera were tested for binding to the HIV-1 IIIB envelope protein. Specific antibody binding to purified HIV-1 gp120 was demonstrated by ELISA using sera obtained from HIV-IT (V) treated mice, whereas pre-bleed, normal mouse sera showed no reactivity. Substantial antibody activity was elicited following the second HIV-IT (V) injection. These results demonstrated the ability of direct administration of HIV-IT (V) to induce HIV-1 IIIBenv-specific antibody.

3.1.3 Conclusion

The preceding experiments have demonstrated the feasibility of employing HIV-IT (V) to induce HIV-1 env-specific CTL and antibody responses in mice. These results established the scientific basis for subsequent studies in non-human primates involving the use of HIV-IT (V). The mouse studies illustrate the proof-of-concept that direct administration of a retroviral vector can result in the induction of HIV-1 env-specific immune responses, including anti-HIV-1 env crossreactive CTL.

3.2 NON-HUMAN PRIMATE MODELS

Preclinical studies employing Rhesus monkeys and baboons were designed to evaluate the direct administration of HIV-IT (V) in non-human primate models. The primate models were utilized to (1) determine the ability of HIV-IT (V) to induce HIV-1 env-specific CTL responses, and (2) to establish an initial dose level, administration route, and sample handling procedures.

Non-human primate studies consisted of two major components: (1) HIV-IT (V) administration; and (2) autologous CTL target cell development. An autologous CTL target cell line was established for each monkey included in the study. These continuous cell lines were initially established by transforming peripheral blood mononuclear cells (PBMC) with *Herpes papio* (HP) or Epstein-Barr (EBV) virus. The established transformed cell lines were subsequently transduced with HIV-IT (V), selected in

geneticin (G418) for neo^r , and monitored for HIV-1 env expression. In this manner, a unique autologous HIV-1 IIIBenv-expressing CTL target cell population was established for each monkey.

3.2.1 CTL Induction

One Rhesus monkey was injected IM with a total of 3×10^7 cfu of purified, formulated HIV-IT (V). Injections were distributed at six sites comprising the upper left and right biceps (0.5 ml/site), and the two sites each for left and right quadriceps (0.5 ml/site). Three complete injections (6 sites/injection) were given at two-week intervals. Blood samples were obtained immediately prior to each injection, and isolated PBMC were restimulated with irradiated autologous HIV-1 env-expressing target cells and after 7 days analyzed for HIV-1 env-specific CTL activity (standard 4 hour ^{51}Cr -release assay). Cytotoxic T-cell activity was demonstrated in the monkey following the first HIV-IT (V) injection (Figure 16) and activity was maintained following a second injection. Therefore, these results indicated that marked CTL activity was induced in a normal Rhesus monkey injected with HIV-IT (V). CTL induction has been demonstrated in six additional Rhesus monkeys, using 6 different vector preparations. Additional studies were carried out in two baboons injected with different vector preparations. Restimulated PBMC from one baboon injected twice IM with a total dose of 3×10^7 cfu of purified, formulated HIV-IT (V) show HIV-1 env-specific CTL activity. Restimulated PBMC from a baboon injected with 3×10^6 cfu did not show CTL activity suggesting that the threshold for CTL induction may be between 3×10^6 to 3×10^7 cfu.

3.2.2 Antibody Induction

Antibody responses specific for the HIV-1 env protein were evaluated in a baboon following one and two IM injections with 1×10^7 cfu purified, formulated HIV-IT (V). Serum samples were obtained following each injection and evaluated for binding to purified HIV-1 gp120 protein-coated microtiter wells by ELISA. Serum

samples obtained following each injection of HIV-IT (V) showed an HIV-1 gp120 specific antibody response as compared to pre-bleed serum. Therefore, the injection of HIV-IT (V) appears to elicit gp120-specific antibodies in a baboon.

3.2.3 Toxicological Assessment

Toxicology studies were performed in Rhesus monkeys given direct administration of a 5×10^7 cfu HIV-IT (V) retroviral vector possessing the same provector as DA/N2 IIIBenv.2C3 but derived from a different canine PCL. Monkeys were evaluated clinically and histologically during and following 4 IM injections of HIV-IT (V). Two groups (6/group) of male and female monkeys were given HIV-IT (V) or diluent control. None of the animals exhibited clinical abnormalities during the 1 year study. In addition, following necropsy and histological preparation of various tissues no pathological lesions were detected. Therefore, the retroviral-vector delivered N2 IIIBenv provector construct did not induce any significant toxicological effects in Rhesus monkeys.

3.2.4 Conclusion

In conclusion, the preceding studies demonstrated the ability of HIV-IT (V) to induce HIV-1 env-specific CTL and antibody responses in non-human primate models. These studies have also demonstrated lack of acute or chronic toxicity in Rhesus monkeys.

3.3 VECTOR LOCALIZATION

Experiments have been initiated to analyze localization of HIV-IT (V) after IM administration. Early studies have focused on analysis of the injection site (muscle), the planned site for human trials, with the aim of demonstrating the *in vivo* appearance of HIV-IT (V) DNA early after vector administration and the subsequent clearance of vector transduced cells over time.

To examine the appearance of HIV-IT (V) DNA at the injection site, eight-week old mice (12 males, 4 females) were injected IM one time with 0.1 ml (1×10^6 cfu) of

purified formulated HIV-IT (V). Mice were sacrificed 24 hours and 72 hours post injection. The injection site, contralateral muscle, spleen, liver, kidneys, thymus and gonads (testes from male, ovaries from female) were removed from each animal at the time of sacrifice and immediately frozen. In preliminary studies, we have examined injection site muscle samples from 8 animals sacrificed 72 hours post-injection with HIV-IT (V) and muscle from a control non-injected animal. HIV-IT (V) DNA was detected by PCR amplification of the HIV-1 env gene in the DNA isolated from injection sites taken from seven of the mice (Figure 17). DNA was not recovered from the final animal in this group. Amplified product was not detected in reactions containing DNA isolated from the muscle taken from the negative control (non-injected) mouse. The sensitivity of the assay is estimated at 1 copy per 10^5 cells from separately performed spiking experiments. Individual experiments were controlled by spiking 2.5 and/or 5 copies per 10^5 cell equivalent(s) of DNA. These results show we can detect HIV-1 env sequences encoded by HIV-IT (V) at the injection site.

To determine the time course of the appearance of HIV-1 env DNA and its subsequent clearance, tissue samples from HIV-IT (V) injected mice were obtained at various timepoints after to vector administration. In a separate study, four groups of five male mice were injected IM one time with either diluent control (1 mouse per group) or with 0.1 ml (1×10^6 cfu) of purified, formulated HIV-IT(V) (4 mice per group). One group (4 experimental, 1 control) of mice was sacrificed at each time point post-injection: 2 hours, 24 hours, 7 days or 21 days. The injection site, contralateral muscle, spleen, liver, kidneys, thymus and gonads were removed from each animal at the time of sacrifice and immediately flash frozen. The presence of HIV-IT provector was determined using PCR amplification of the HIV-1 env gene in DNA isolated from the injection sites taken from mice sacrificed at each timepoint.

The results indicate that transduction of cells at the site of injection is detectable after 2 hours in four out of four mice tested. *In vitro* studies of the retroviral life cycle indicate

that 6 - 24 hours is normally required for absorption, reverse transcription, and integration. Because of the nature of the PCR assay, it is unclear what proportion of the potentially transducible cells contain HIV-1 env DNA at each of the timepoints. The positive signal may represent as few as 2.5 transduced cells in the sample, because the assay can detect at least 2.5 copies of HIV-1 env DNA in a muscle preparation containing approximately 10^5 cell equivalents of genomic DNA. The detection of HIV-1 env DNA in the injection sites at day 7 and day 21 indicates that transduction had occurred in these animals. This shows that clearance of transduced cells to levels below 2.5 copies/ 10^5 cell equivalents had not yet occurred for four out of four mice sampled at day 7 or day 21.

3.4 HUMAN *IN VITRO* MODEL

Preclinical studies employing blood samples from HIV-infected individuals were designed to answer several questions about the ability of these individuals to respond to HIV-1 IIIB env epitopes presented by HIV-IT (V) transduced autologous lymphocytes. These questions were:

1. Can HIV-IT (V) transduce human lymphoblastoid cell lines from HIV-1-infected individuals for use as stimulators and targets in CTL assays?
2. Will it be possible to develop a reliable human CTL assay which can be used to monitor human CTL responses to HIV-IT (V)?
3. Will there be pre-existing levels of HIV-1-specific CTL in HIV-1-infected patients?
4. Will pre-existing CTL levels be related to patient CD4 counts?
5. Will *in vitro* human CTL responses be specific to the HIV-1 IIIB strain represented by HIV-IT (V) or will crossreactivity be observed, comparable to that exhibited by the mouse CTL on HIV-1 infected human targets (Section 3.1.1.6)?

To attempt to evaluate the biological effect of HIV-IT (V) on CTL activity in HIV infected humans, an *in vitro* system was developed to reliably measure HIV-1 specific CTL. Autologous Epstein-Barr virus (EBV) transformed cell lines were derived from

each of 32 HIV-positive patients such that target/stimulators for each patient had the correct MHC for antigen presentation. These EBV-transformed cell lines were transduced with HIV-IT (V), and tested by Western blot analyses to verify HIV-1 env expression. These cell lines were also transduced with an HIV-1 gag expressing vector. PBMC were isolated from whole blood samples of patients by a Ficoll-Hypaque gradient. Isolated PBMC were cultured with irradiated autologous HIV-1 env-expressing EBV-transformed cell lines for 7 to 10 days. After this *in vitro* stimulation, the cultures were used as effectors in a ^{51}Cr -release assay where net specific lysis of HIV-1 env-expressing cells was measured.

Most adults have been exposed to EBV, thus, when the patient PBMC are stimulated *in vitro* with an EBV-transformed cell line, the EBV-specific CTL are efficiently activated. Two approaches were taken to address this issue. First, differing ratios of responder PBMC to EBV stimulator cells (R:S ratio) were used in the *in vitro* culture step which lowers background lysis. Secondly, excess unlabeled EBV-targets were incorporated into the assay to compete out the EBV-specific lysis of the labeled target. Both approaches proved to be useful in controlling the masking effect that EBV-specific lysis exhibited.

Thirty two patients with CD4⁺ counts from 260 to greater than 1,000 were tested for HIV-1 env-specific lysis after *in vitro* stimulation. Twenty six patients had a net specific lysis greater than 20%, 5 had net specific lysis between 10 - 20% only one patient consistently had no HIV-1 env-specific lysis (net lysis less than 10% at 10 different time points). Thirty-one of these patients have been tested for HIV-1 gag responses, with 30 demonstrating net specific lysis greater than 20%. Additional experiments indicated that the measured lysis was not attributable to natural killer cell activity. In a single patient, the CTL were shown to be CD8⁺. Further characterization of the CD4⁺ and CD8⁺ components of this CTL response in humans is underway.

The CTL assay is based on a complex biological system, with consequent variability in the reproducibility of the results. Therefore, studies have been undertaken to determine the variability of the human HIV-1 env ^{51}Cr -release assay. Preliminary data indicate that the coefficient of variation from individual measurements is between 10 - 20%, depending on the effector to target cell ratio that is being evaluated. This makes the assay possible to work with, but the variability and the relatively high level of HIV-1 env-specific CTL detected following restimulation of patient PBMC point to the potential advantage of a more HIV-IT (V)-specific marker of CTL activity. One approach would be to measure the HIV-1 env-specific lysis without the *in vitro* stimulation step resulting in lower background lysis (but also lower HIV-1 env-specific lysis). In 5 patients studied thus far, only one patient showed direct lysis above 10% (Figure 18), whereas all 5 exhibited levels of lysis considerably above 10% after *in vitro* stimulation. Because measurable CTL in the direct assay is a rare event, administration of HIV-IT (V) might boost the level of CTL activity measured directly to significant levels of lysis above that obtained prior to HIV-IT (V) administration.

An additional approach to examining responses to HIV-IT (V) involves use of peptide coated targets. Studies were initiated to examine the lysis of HIV-1 IIIBenv peptide (P18IIIB) coated target cells. Because HIV-1 IIIB is under-represented in the United States population of HIV-1 infected patients, and the HIV-IT (V) incorporates the IIIBenv gene, responses against the P18IIIB peptide in clinical trial patients would likely be due to HIV-IT (V) stimulation. In pilot experiments, HIV-1 gag peptides employed as positive controls yielded greater than 50% specific lysis in CTL assays using *in vitro* stimulation of PBMC. However, none of the 7 patients tested for P18IIIB specific lysis have exhibited lysis above 5%. Four of these 7 patients were also tested for P18MN specific lysis with only one showing P18MN specific cell lysis (Figure 19). These data suggest that P18IIIB specific peptide lysis may be a useful indicator for HIV-IT (V) induced reactivity.

3.4.1 Regarding human PBMC-derived CTL, the following statements can be made:

- (1) HIV-IT (V) can transduce human lymphoblastoid cell lines derived from HIV infected individuals and these transduced cells can present HIV-1 IIIB antigen for recognition in each of the three related *in vitro* CTL assays described above.
- (2) Measurement of human CTL responses with these assays is feasible, and one or more is likely to have utility in monitoring human clinical responses to HIV-IT (V).
- (3) All HIV-infected patients studied to date have measurable endogenous levels of CTL activity which have no obvious correlation with their CD4 counts.
- (4) All HIV-infected patients with CD4 counts of 260 and above studied to date exhibit CTL activation *in vitro*.
- (5) *In vitro* stimulation of CTL responses with HIV-IT (V)-transduced autologous cells presenting HIV-1 IIIB env epitopes is effective. This appears to represent a recognition of CTL elicited *in vivo* by unknown HIV-1 strains infecting these patients. This suggests by analogy that the CTL crossreactivity between HIV-1 strains seen in the mouse CTL/human target system (Section 3.1.1.6) applies also to the CTL existing within HIV-infected patients.

3.4.2 Conclusion

Preclinical evaluation of the HIV-1 IIIBenv/rev retroviral vector (N2 IIIBenv) has demonstrated it to be free of adventitious agents, to be biologically active both *in vivo* in eliciting CTL both in mice and in primate models, and *in vitro* in generating both stimulator and target cells for use in assays of CTL from HIV-1-infected patients. Evidence obtained from the human CTL assay using stimulated patient PBMC as effectors demonstrates provocative evidence for cross-activation of human CTL by HIV-IT (V). This *in vitro* demonstration that HIV-IT (V) can reactivate human

HIV-specific CTL provides the impetus for *in vivo* administration of HIV-IT (V) as provided for in the attached clinical protocol.

4.0 CLINICAL PROTOCOL (HIV-I-02-1292)

**Title: A Double Blind, Controlled, Dose Escalating Phase I Clinical Trial
to Evaluate the Safety and Biological Activity of HIV-IT (V) (HIV-
1 IIIBenv-Vector) in Asymptomatic HIV-1 Infected Subjects**

U.S. IND No.:

BB-_____

Clinical Study No.:

HIV-I-02-1292

Phase of Study:

Phase I

Date of Protocol:

2/01/93

Protocol Version:

Protocol Version 1.4

Principal Investigator:

Jeffrey E. Galpin, M.D.

Co-Investigator:

Dennis A. Casciato, M.D.

Site of Study:

Shared Medical Research Foundation
5620 Wilbur Avenue, Suite 333
Tarzana, CA 91356

PROTOCOL VERSION 1.4

IND BB _____

Title:

A Double Blind, Controlled, Dose Escalating Phase I Clinical Trial to Evaluate the Safety and Biological Activity of HIV-IT (V) (HIV-1_{IIIB} *env*-Vector) in Asymptomatic HIV-1 Infected Subjects.

4.1 Objectives

4.1.1 In a Controlled, Dose Escalating, Phase I Study of Asymptomatic HIV-1 Infected Subjects to Determine:

- 4.1.1.1 The safety and tolerability of administering three monthly doses of HIV-IT (V) at each of two different dose levels, and
- 4.1.1.2 The pharmacokinetics of the neomycin resistance (*neo^r*) marker gene in successive blood samples.

4.1.2 Secondary Objectives: In these Same Subjects, to Collect Preliminary Data On:

- 4.1.2.1 The pharmacodynamics of the cytotoxic T lymphocyte (CTL) response both *in vitro* and *in vivo*.
- 4.1.2.2 The pharmacodynamics of anti-HIV-1_{IIIB} specific gp120 and p24 antibodies.
- 4.1.2.3 Measurable effects on viral load.
- 4.1.2.4 Measurable effects on T-cell subpopulations and other selected surrogate markers.
- 4.1.2.5 Measurable preliminary indications of possible effects on disease progress.

4.2 Background and Purpose

4.2.1 HIV-1 and the Immune System

- 4.2.1.1 Although nearly all HIV-1 infected persons develop antibodies, such antibodies appear to be unable to clear the HIV-1 infection¹. Most HIV-1 vaccines and immunotherapeutics studied to date have generated HIV-1 reactive antibodies, sometimes at high titers, but there is no published evidence that these antibody responses prevent disease progression.
- 4.2.1.2 There is strong evidence that the HIV-1 virus may be directly transmitted from infected to non-infected cells through several mechanisms, including syncytia formation, without entering the extracellular space². In this circumstance, HIV-1 reactive antibodies have no opportunity to intercept the HIV-1 virus. For this reason, there is a broad consensus among HIV-1 researchers and clinicians that a serious effort to develop HIV-1 reactive cytotoxic T lymphocytes (CTL) represents a largely unexplored and potentially crucial frontier in controlling the progression to AIDS³.
- 4.2.1.3 HIV-1 is a virus which has evolved the ability to evade and confound the immune system as follows: (1) Gene segments coding for its envelope protein mutate frequently, including those portions of the envelope which are immunodominant with respect to antibody production⁴ and, (2) HIV-1 may down-regulate the concentration of major histocompatibility complex (MHC) molecules on the surface of cells in which HIV-1 virions are replicating *in vitro*⁵. If MHC down regulation is also present *in vivo*, this may be responsible for lower levels of CTL induction, since MHC molecules present foreign antigens to the immune system, (3) HIV-1 infects and disables CD4 T-cells, which results in the progressive functional loss of this immune cell population as the infection spreads⁶. The consequence of this loss of

¹Weiss, et al, *Nature*, 316:69-72 (1989)

²Popovic, M., et al, *Science*, 224:497-500 (1984).

³Walker, B.D., et al, *AIDS*, 4:177-184 (1990).

⁴La Rosa, G. J., et al, *Science*, 249:932-935 (1990).

⁵Scheppler, J.A., et al, *J. Clin. Invest.*, 72:398 (1983).

⁶Rosenberg, Z.F., et al, *AIDS Res. and Human Retroviruses*, 5:104 (1989).

CD4 cells is profound immunosuppression, leaving the patient unprotected against opportunistic infections and cancers, which cause the morbidity and mortality of HIV infection.

- 4.2.1.4 Given these tactics by the virus, it is not altogether surprising that the therapeutic contributions made by humoral and cellular immunity in the control of the pathologic effects of HIV-1 infection are poorly understood. HIV-1 specific antibodies, some of which possess *in vitro* neutralizing activity⁷, are generated during the natural course of HIV-1 infection. Neutralizing antibodies may eliminate extracellular virus in vivo, but HIV-1 can also spread by cell-to-cell fusion. Thus HIV infection and spread may continue in the presence of antibody. Epidemiologic studies and preliminary investigations using passive immunotherapy further confirm that clinical disease and infectivity can be only partially delayed by even high levels of neutralizing antibody.
- 4.2.1.5 T-cell mediated immunity, involving the induction of CD8⁺ CTL that directly destroy virus-infected cells, has been shown to be important in limiting the severity and duration of several viral diseases⁸. CD8⁺ CTL, capable of lysing target cells expressing HIV-1 envelope, gag, polymerase, and nef proteins have been detected in HIV-1 seropositive patients, and may be important in controlling HIV-1 infection⁹. Although the precise impact of the immune response on HIV-1 infection remains to be elucidated, it is likely that the viral infection and replication process does not permit the full therapeutic benefit of the immune response to be invoked.

4.2.2 Rationale for an HIV-1 Gene Transfer Immunotherapeutic

- 4.2.2.1 Given these considerations, the rationale for use of HIV-IT (V) is: (1) To attempt to stimulate the immune system, particularly CD8⁺ CTL induction, more broadly and to a more vigorous level than is allowed by the natural virus infection, and (2) To provide this immune stimulation as early as possible post-HIV-1 infection, thereby mobilizing the

⁷Robert-Guroff, M., et al, *Nature*, 316:72-74 (1985)

⁸Rouse, R.T., *Rev. Infect. Dis.*, 10:16-33 (1988).

⁹Koenig, S., et al, *J. Immunol.* 145:127-135 (1990).

immune system before it becomes severely compromised by HIV-1 disease.

- 4.2.2.2 Generally, a long, symptomless period follows the initial acute infection with HIV-1¹⁰. Infected individuals could, therefore, benefit from an immunotherapy that stimulates both potent HIV-1 specific CD8⁺ CTL, neutralizing antibodies, or both. CD8⁺ CTL are extremely potent "killers" of virally infected cells¹¹. The Class I MHC restricted CD8⁺ CTL system is the arm of the immune system that is most specifically designed to combat viral infections¹². HIV-IT (V) is a promising agent for clinical trials as a treatment in HIV-1 infection because cells transduced with HIV-IT (V) have been shown to specifically induce the CTL immune response of mice and macaques at doses that lack appreciable toxic side effects¹³. Although not yet administered clinically, Viagene has data indicating that HIV-IT (V) treated autologous cells can stimulate a CD8⁺ human CTL response *in vitro*, (using PBMC obtained from HIV-1 seropositive patients). In addition, HIV-IT (V)-treated mice can also elicit CD8⁺ CTL that selectively destroy cells expressing HIV-1 proteins *in vitro*. HIV-IT (V) also elicits a concomitant antibody response in the murine system directed against the HIV-1 III_B envelope protein.

4.2.3 Description of HIV-IT (V)

- 4.2.3.1 HIV-IT (V) is an intramuscularly (IM) injected HIV-1 immunotherapeutic based on retroviral vector-mediated gene transfer technology that provides for the delivery of the *env* gene segment of the HIV-1 III_B strain directly into patient cells. The retroviral vector containing the gene is subjected to Quality Control testing, and injected into multiple IM sites. Proteins expressed from this gene and presented on the surface of transduced recipient cells may cause significant stimulation of immune responses, and in particular, the CD8⁺ CTL response. An overview of the gene transfer procedure used to generate HIV-IT (V) is

¹⁰Robert-Guroff, M., et al, *Nature*, 316:72-74 (1985).

¹¹Klavinskis, L.S., et al, *J. Virol.*, 63:4311-4316 (1988).

¹²Koenig, S., et al, *J. Immunol.*, 145:127-135 (1990).

¹³Warner J.F. et al, *AIDS Res. & Human Retroviruses*, 7:645-655, (1991)

shown in Figure 1: Gene Transfer-Based Immunotherapeutics: HIV-IT (V).

4.2.4 Summary of Preclinical Research and Toxicology/ Pharmacology Studies

- 4.2.4.1 Beginning in September, 1990, Viagene and the scientific staff at the FDA's Center for Biologics (CBER) worked jointly to develop a comprehensive toxicology/pharmacology plan to evaluate HIV-IT (V). Studies were designed for Rhesus macaques monkeys and mice in order to address issues of safety, toxicity, potential tumorigenicity, and biologic activity.
- 4.2.4.2 Initial preclinical pharmacology studies were conducted in a mouse model. The mouse system has been used to evaluate the induction of humoral and cellular immune responses due to direct administration of HIV-IT (V) or syngeneic cells transduced with HIV-IT (V). Briefly, mice were immunized with a series of HIV-IT (V) injections and anti-HIV-1 ENV specific CD8⁺ CTL responses were analyzed (Figure 2). HIV-IT-treated murine cells injected into mice induced a strong CTL response directed against target cells expressing the HIV-1 III_B envelope protein (BCenv), whereas no significant lysis of the control BC cells was observed.
- 4.2.4.3 HIV-IT (V) has consistently elicited substantial CTL responses in mice. In this model system, multiple immune response parameters have been examined in detail. These include (1) single vs. multiple injections, (2) route of administration, (3) phenotype of the effector population, (4) MHC restriction, and (5) the specificity of the immune response with regard to ENV, REV and NEO^R determinants.
- 4.2.4.4 Although HIV-IT (V)-transduced cells express the env gene from HIV-1 III_B it is known that the HIV-MN prototypic laboratory viral strain is a more serologically representative strain in patients¹⁴. Data are presented in Figure 3 demonstrating that mice immunized with HIV-IT (V) encoding the env gene from the laboratory strain HIV-1 III_B induce CTL which recognize and lyse cells expressing not only the homologous HIV-1 III_B strain, but also lyse cells coated with a peptide

¹⁴Devash, Y., et al, *AIDS Res. & Human Retroviruses*, 6:307-316 (1990).

(P18IIIB) derived from the V3 region of the HIV-1IIIB envelope protein. The HIV-IT (V)-induced CTL also lyse target cells coated with the P18MN peptide derived from the equivalent V3 region of the HIV-1MN envelope protein. Similar CTL crossreactivity directed against proteins of different HIV-1 isolates has been demonstrated following immunization of mice with the syngeneic cells transduced with HIV-IT (V). Thus, the CTL response appears to exhibit broad reactivity or clinical coverage against diverse HIV-1 strains. Mice treated with HIV-IT (V) have also produced antibodies that specifically react to the HIV-1IIIB envelope protein. Mice were immunized by intramuscular injections several times and their serum tested for reactivity (binding) to gp120 (Figure 4).

- 4.2.4.5 Rhesus macaques have been immunized with HIV-IT (V), resulting in the production of HIV-1 specific CTL. The intramuscular administration of successive doses of HIV-IT (V) to Rhesus macaques has resulted in the development of HIV-1 ENV specific CTL responses. Data from a treated macaque are shown in Figure 5.
- 4.2.4.6 Blood from HIV-1 infected patients has been examined to see whether anti-HIV-1 specific CTL responses could be amplified using HIV-IT (V) treated autologous cells. Figure 6 shows that PBMC from an HIV-1 infected individual can be reliably restimulated by HIV-IT (V) transduced autologous cells in order to expand the number and potency of anti-HIV-1 ENV specific CD8⁺ CTL *in vitro*. Restimulated CTL from the patient lyse autologous cells expressing HIV-1IIIB env. However, the restimulated cells do not lyse non-transduced patient cells (control). These data support the view that human CD8⁺ CTL exist in HIV-1 infected individuals and can be stimulated to increased activation by HIV-IT transduced autologous cells. It will be important to ascertain whether a similar triggering phenomenon can occur *in vivo*.
- 4.2.4.7 In summary, preclinical data support the view that HIV-IT (V) can be safely administered to both mice and non-human primates, that strong CD8⁺ CTL and antibody responses can be induced in animal models, that cross-reactive immune responses between divergent HIV strains have been demonstrated by HIV-IT (V) induced murine CTL, and that HIV-IT (V) transduced cells can amplify *in vitro*, pre-existing CD8⁺

CTL obtained from HIV-1 infected individuals. These data provide the impetus to initiate studies of HIV-IT (V) in humans. Based on the successful production of CTL responses achieved in Rhesus macaques that received three doses of HIV-IT (V) given at 2 week intervals and on a careful analysis of these data, Phase I clinical trial subjects will receive three doses of HIV-IT (V) at monthly intervals given in two successively escalating dosage groups (see Section 4.4.3). Control subjects will receive a placebo comprised of HIV-IT (V) formulation media. After an appropriate evaluation interval, these control subjects may have the option of receiving the highest tolerated dose of HIV-IT (V).

4.3 Patient Eligibility For the Provisional (Pre-treatment) Phase of Study

4.3.1 The Following Criteria are Required for Subjects to be Eligible for Provisional Admission. All Candidate Subjects Must:

- 4.3.1.1 Be HIV-1 infected as demonstrated by a confirmed positive test for HIV-1 antibodies, an HIV-1 positive virus culture, or a positive test for circulating p24 antigen.
- 4.3.1.2 Have two or more baseline CD4⁺ T-cell counts ≥ 400 or an absolute CD4⁺ T-cell percentage of $\geq 20\%$ which have been obtained at least 28 days apart, with the most recent count obtained within the last 30 days.
- 4.3.1.3 Have a performance status of $\geq 80\%$ on the Karnofsky Scale, or equivalent.
- 4.3.1.4 Be an adult male or female (age 18 to 65). Both male and female subjects must use barrier birth control measures.
- 4.3.1.5 Have acceptable clinical chemistry and hematology laboratory findings within the normal range, including hematocrit $\geq 34\%$ for men and $\geq 30\%$ for women, hemoglobin ≥ 10 g/dl, granulocytes ≥ 1500 cells per mm³, platelet count $\geq 75,000$ per mm³, SGOT (AST) ≤ 3 the upper limit of normal, SGPT (ALT) ≤ 3 the upper limit of normal, creatinine ≤ 2 the upper limit of normal or a creatinine clearance of ≥ 50 ml per minute.
- 4.3.1.6 Render an Informed Consent in accordance with institutional policies.

4.3.2 The Following Criteria Will Make Subjects Ineligible for Entry into this Study:

- 4.3.2.1 Pregnant or lactating females, and all other persons of both sexes with or without reproductive potential who do not use barrier birth control measures adequate to prevent HIV transmission.
- 4.3.2.2 Active drug or alcohol abuse.
- 4.3.2.3 Candidates who have received anti-neoplastic chemotherapy within six months of entering the study, candidates who have received non-HIV related experimental therapy within 45 days of entering the study, candidates who, within 45 days of commencing treatment, have received anti-retroviral therapy, and candidates who, within four weeks of commencing treatment, have received therapy with immunomodulators or corticosteroids.
- 4.3.2.4 Subjects who have previously received an anti-HIV-1 immunotherapeutic or vaccine, unless such an anti-HIV-1 product has become approved and is universally recommended for HIV-1 positive subjects.
- 4.3.2.5 Subjects who currently have or develop, during the baseline of observation or before crossover, any HIV-1 related opportunistic infection, malignancy or AIDS defining event involving any system. Exceptions are *Herpes zoster*, *Herpes simplex*, psoriasis, seborrheic dermatitis, sinusitis and lymphadenopathy.
- 4.3.2.6 Subjects who have received a major organ allograft or who have significant cardiac, pulmonary, autoimmune, renal or central nervous system disease or malignancy.
- 4.3.2.7 Subjects identified as anergic by delayed type cutaneous hypersensitivity (DTHT) testing. DTHT testing will employ at least four recall antigens. If the erythematous response invoked by every test antigen is less than 2 mm in diameter, the subject will be considered anergic.

4.4. Study Plan

4.4.1 HIV-IT (V) Phase I Study Schema

Informed Consent	Clinical History & Physical Exam	Prepare target cells for CTL assays
Initial Screening	Baseline blood and urine tests	

Formal Admission

<u>Dosage Group I</u> First Dosage Level; HIV-IT (V) on Days 0, 28 and 56 (concurrent with Control Group)	Follow-up at Day 70	Safety Evaluation
<u>Control Group I</u> Placebo on Days 0, 28 and 56	Follow-up at Day 70	Safety Evaluation
<u>Dosage Group II</u> Second Dosage Level HIV-IT (V) on Days 0, 28 and 56	Follow-up at Day 70	Safety Evaluation
<u>Control Group-Crossover</u> Highest well tolerated dose of HIV-IT (V) on Days 0, 28 and 56.	Follow-up at Day 70	Safety Evaluation; Sponsor's Full Initial Evaluation and Study Reports to Regulatory Agencies

Follow-up

<u>Continuing follow-up</u> Month 12	Sponsor's Mid-Term Evaluation	Sponsor's Interim Report(s) to Regulatory Agencies
<u>Long-Term Follow-Up</u> (Up to 3 years at 6 month intervals)	Sponsor's Long-Term Evaluation	Sponsor's Interim and Final Report(s) to Regulatory Agencies

TABLE I

PHASE I DOSE ESCALATION STUDY OF HIV-IT (V)

Minimum # of Subjects		Product Administration: Cycle I		Product Administration: Cycle II (Crossover)
Stage 1	5	10 ⁶ HIV-IT (V) cfu		
	5	Placebo Control	X	10 ⁷ HIV-IT (V) cfu ¹
Stage 2	5	10 ⁷ HIV-IT (V) cfu (Control)		
15 Subjects				

¹All control subjects will be provided the opportunity for crossover to the highest well tolerated dose of HIV-IT (V).

No control crossover injections will be initiated until at least three of the Stage 2 (highest dose) HIV-IT (V) recipients have been evaluated for at least two weeks after their third injection.

4.4.2 General Plan for Provisional Admission

- 4.4.2.1 After completion of the screening evaluation and documentation of Informed Consent, qualified subjects will be admitted into this clinical study. Admission requires that: (1) the subjects meet all entrance criteria, and (2) the subject's peripheral blood leukocytes transform, transduce and propagate satisfactorily for CTL target cell development.
- 4.4.2.2 A portion of the 100 ml sample of blood will be used to generate an immortalized, autologous B cell line. The immortalized cells are transduced with a retroviral vector encoding the HIV-1III_B *env*, *rev* and the *neo^r* genes. These cells are then used as *in vitro* stimulators and as immunological targets in the CTL assay. Additional laboratory tests may be performed on this and other blood samples to verify a subject's continued eligibility for the study.

4.4.3 General Plan for Formal Admission and Treatment Phase of the Study

- 4.4.3.1 The clinical study will proceed in two successive stages represented by two HIV-IT (V) escalating dose groups. These two stages will consist

of doses of one million and 10 million HIV-IT (V) cfu, respectively. The first HIV-IT (V) treatment group will be paralleled by a concurrent placebo control group. All placebo control subjects will be provided the opportunity for crossover to the highest well tolerated dose of HIV-IT (V).

- 4.4.3.2 Subjects will be randomly assigned to HIV-IT (V) and control groups at the time of their formal admission to the study. Control subjects will have the option to cross-over to the highest well tolerated dose of HIV-IT (V) after at least three subjects in the high dose group have been evaluated for two weeks beyond their final HIV-IT (V) injections.
- 4.4.3.3 Each subject in the HIV-IT (V) groups and in the placebo control group will receive four 0.5 ml product injections IM on each injection day. Injections will be made bilaterally into the deltoid and into the gluteus maximus.
- 4.4.3.4 The first dosage group will complete all three injections, given at 28 day intervals, followed by a two-week safety evaluation. Thereafter HIV-IT (V) will be administered to the higher dosage group.
- 4.4.3.5 Safety will be evaluated by physical examination, interim clinical history, and laboratory analyses. Any deviations from normal will be scored according to the BRMP toxicity scale included in Section 4.14.0.
- 4.4.3.6 The minimum number of patients for each of the three subject groups in this study is provided in Table I (4.4.1).

4.4.4 Summary of Blood and Urine Samples to be Collected from all Subjects for Both Routine and Specialized Laboratory Tests

- 4.4.4.1 Blood samples will be drawn at Days -4 and 24 to assess by PCR possible migration of the *neo^r* marker gene. On follow-up Day 70 and Month 12, further blood samples will be drawn and assessed by PCR to detect evidence of persistence or spread of the NEO^R marker from HIV-IT (V). Additional blood samples will be drawn at Days -4, 12, 24, 36, 52, and during follow-up at Days 70 and 84, and Months 6, 12, 18, 24, 30 and 36 to assess safety and tolerability, to measure the immunostimulatory effect of HIV-IT (V), and to identify changes in

potential surrogate measures of outcome. See the Subject Schedule (Section 4.13.0).

- 4.4.4.2 Urine samples will be collected prior to injection on Treatment Days -4, 24, and 52, and on follow-up Day 70 and Months 6, 12, 18, 24, 30 and 36.

4.4.5 Sample Shipment Instructions

- 4.4.5.1 Whole blood and serum samples will be labeled with the appropriate subject identification number, time and date of collection, wrapped in absorbent material and packaged in leak-proof, crush-resistant mailers following standard bio-hazard materials transportation regulations. All samples will be transported immediately by courier to Viagene's BioSafety Level 3 laboratory or to contracted vendor laboratories for processing.

4.5. Formal Admission and Evaluation During the Treatment Phase of the Study

- 4.5.1 To be eligible for Cycle I product administration (see Table I), subjects must have a reconfirmed, mean, CD4⁺ T-cell count that is either ≥ 400 or $\geq 20\%$ or approaches either of these values and is within the range of experimental error for the laboratory performing the CD4 assay. Subjects must continue to meet the inclusion and exclusion criteria as stipulated in Sections 4.3.1 and 4.3.2.
- 4.5.2 To be eligible for crossover administration of HIV-IT (V), control subjects must not have developed any AIDS defining conditions, although their CD4⁺ T-cell counts may have dropped below 400. Subjects must continue to meet all the inclusion and exclusion criteria as stipulated in Sections 4.3.1 and 4.3.2.
- 4.5.3 Subjects will be required to provide a blood sample 4 days prior to each injection day.
- 4.5.4 A chem profile, CBC/diff, platelet count, and urinalysis will be performed on Days -4, 12, 24, 36, and 52, and on follow-up Day 70, and Months 6, 12, 18, 24, 30 and 36. An interim clinical history and relevant physical examination will be performed on Days 28 and 56 to detect the occurrence of any adverse reactions since the previous injection, and on follow-up Days 70 and 84 and Months 6, 12, 18, 24, 30 and 36.

- 4.5.5. The cumulative incidence rate of a set of early signs and symptoms of disease, termed Clinical Event Markers, will be developed from data obtained from the interim history records. This will provide a preliminary indication of possible effects on disease progress and will serve as another indicator of safety. These markers will include HIV-related symptoms as defined by CDC classification (and in addition *Candida* infections, *Herpes zoster*, hairy leukoplakia, seborrheic dermatitis, sinusitis, lymphadenopathy, and weight loss). These measures will be taken on Days -4, 28 and 56 prior to injection, and on follow-up Days 70 and 84, and Months 6 and 12.
- 4.5.6 A blood sample will be obtained for measurement of circulating CTL at Viagene's laboratory, employing an experimental ^{51}Cr release assay, on Days -32, -18, -4, 12, 24, 36, and 52 and on follow-up Days 70 and 84, and Months 6 and 12.
- 4.5.7 T-cell subset assays will be performed on Days -90, -18, -4, 24, and 52, and on follow-up Days 70 and 84, and Months 6, 12, 18, 24, 30 and 36.
- 4.5.8 A blood sample for HIV-1 gp120 ELISA antibody assay will be obtained on Days -90, -32, 12, 24, 36 and 52, and on follow-up Day 70, and Months 6, 12, 24, and 36. Additionally, assays for anti-retroviral drugs may be performed at any time during treatment and follow-up at the discretion of the Investigators.
- 4.5.9 A blood sample for measuring leukocyte viral load and other biological assays (potential surrogate markers) will be obtained on Days -90, -32, -4, 12 and 52 (prior to injection), and on follow-up Day 70, and Months 6, 12, 24 and 36. Leukocyte viral load will be determined by p24 antigen levels following cocultivation of subject PBMC. An experimental assay employing PCR for HIV nucleotide sequences may also be used.
- 4.5.9.1 The selection of assays to be performed on a subject's blood sample at any given time point will depend on (1) specimen availability, (2) whether an assay(s) under development at Viagene has(have) been established for use, and (3) predetermined assay priorities. The assays have been grouped into three categories and, reflecting the trial's emphasis on safety, have been prioritized as follows:
- (1) Patient Health & Safety (e.g. clinical chemistry and hematology panels, CD4^+ T lymphocyte counts),
 - (2) HIV Disease Status (e.g. CD4^+ T-cell counts, viral burden),
 - (3) Biological Activity (e.g. CTL, and antibody assays).

- 4.5.10 In order to assess the pharmacokinetics of the *neo^r* marker by PCR at Viagene's laboratory, a 5 ml heparinized blood sample will be obtained on Days -4 and 24 for participants in each treatment cycle. PCR assay for *neo^r* on blood samples taken at follow-up Day 70 and Month 12 will also be performed to detect evidence of persistence or spread of HIV-IT (V). See Subject Schedule (Section 4.13.0).
- 4.5.10.1 Designated blood samples will be shipped to Viagene according to the specific procedures to be provided.
- 4.5.11 The subject will be observed continuously for the first fifteen minutes following each injection and at 30, 60, and 120 minutes thereafter. Vital signs (BP, P, T, R) will be obtained prior to each injection and at 15, 30, 60 and 120 minutes following each injection.
- 4.5.12 Adverse reactions will be noted on the Adverse Reaction Case Report Form as they occur. The Baseline Symptom Checklist will be completed on entering the study and before the first injection.

4.6 Plan of Analysis

- 4.6.1 Data collected during this Phase I study will include the following variables: clinical history and physical examination parameters, vital signs, report of adverse events, chemistry profile, CBC and differential, platelet count, urinalysis, CD4⁺ T-cell count, HIV antibody titer, viral load, CTL response, *neo^r* in blood by PCR, and selected surrogate markers. These variables will be measured at multiple time periods. Findings from this study will be tabulated and presented, and clinically meaningful results will be analyzed. For example, a drop of 40% in CD4⁺ T-cell count from the formal admission level might indicate a safety issue to be investigated. Likewise, all out of range laboratory data will also be addressed.
- 4.6.2 Pre-treatment versus post-treatment comparisons of selected continuous variables will be made for individual HIV-IT (V) dosage groups and the Control Group, and for both HIV-IT (V) dosage groups combined versus the Control Group using analyses of variance (ANOVAs). Pre-treatment versus post-treatment comparisons of selected categorical variables will be made for the Control Group and individual HIV-IT (V) dosage groups combined using Fisher's Exact tests or categorical log-linear models.

4.7 Risks and Discomforts

Risks associated with blood drawing may include transient pain, bruising or lightheadedness, and a modest risk of infection. Risks associated with product injections include local pain, inflammation, erythema, swelling, itching, tenderness, skin discoloration, skin breakdown, regional lymphadenopathy, and possibly even granuloma formation at the injection site. Serious inflammation is not anticipated; however, should this occur, symptomatic treatment will be provided. Other clinical manifestations that could result from product administration include fever and chills, diaphoresis, mild gastrointestinal distress, allergic reactions which may include rash, decrease in blood pressure, shortness of breath and/or syncope, and remotely, anaphylaxis.

There are several theoretical safety concerns related to the use of retrovirally vector-mediated gene transfer¹⁵. These are summarized in Section 4.7.1 through 4.7.5.

4.7.1 Production of Replication Competent Virus Through Recombination

4.7.1.1 The risk of producing replication-competent retrovirus through recombination is remote since (1) All known human endogenous retroviruses have multiple disruptions in their coding sequences¹⁶, and Southern blot analysis of human genomic DNA, probed with the HIV-1 *env* gene, has failed to demonstrate any significant homology, (2) The canine packaging cell utilized in HIV-IT (V) production has been engineered to minimize the possibility of generating replication-competent retrovirus¹⁷; (3) A split genome approach to produce the packaging cell line predicts that three separate recombination events would be required in order to produce a replication competent murine virus¹⁸, (4) Quality Control testing using the S⁺L⁻ extended assay to detect amphotropic and xenotropic retroviruses will be performed on HIV-IT (V) prior to Quality Control release for injection¹⁹.

¹⁵Cornetta K. et al, *Human Gene Therapy*, 2:5-14, 5-15, (1991).

¹⁶Abraham, G.N. and Khan, A.S., *Clin. Immunol. & Immunopath.*, 56:1-8 (1990).

¹⁷See Drug Master File # BB-MF4452, Section IV.B.3.b. (p.57) and IV.B.4.c.(5)(a) (p69).

¹⁸Miller, D.A., *Human Gene Therapy*, 1:5-14 (1990).

¹⁹Bassin, R.H., et al, *Int. J. Cancer*, 6:95-107 (1970).

4.7.2 Malignancy Caused by Insertional Mutagenesis

This is a non-trivial risk. From a theoretical standpoint, approximately four to five insertional events are likely required within a cell to cause malignant transformation²⁰. In this case, the risk of potential malignant transformation has been assessed by tumorigenicity studies conducted in nude mice, immunosuppressed suckling mice, and transformation studies in soft agar. No evidence of tumor formation in the former (nude and suckling mice) or transformation in the latter [soft agar using cells transduced with HIV-IT (V)] has been observed.

4.7.3 Contamination and/or Mutation of Germ Cells

This risk is largely theoretical because (1) the vector is engineered to be non-replicating; and (2) extensive PCR analysis of male Rhesus macaque monkeys and HIV-1 infected chimpanzees after treatment with HIV-IT (V) has shown no transfer of HIV-IT (V) into the germ line tissues of these animals.

4.7.4 Further Immune Suppression Precipitated by the Release of Active Infectious HIV-1 Virus From CTL-Lysed HIV-Infected Macrophages

- 4.7.4.1 The magnitude of this risk is uncertain, however, most intracellular HIV-1 is not infectious, and becomes infectious only after budding out from cell surfaces.
- 4.7.4.2 Clinical and laboratory parameters will be monitored for evidence of exacerbation of disease.
- 4.7.4.3 CD4⁺ T-cell will be monitored in samples taken 2 days prior to injection and at each visit thereafter. Viral burden will be monitored frequently.

4.7.5 Exacerbation of Disease Due to the Killing of Uninfected CD4 T-Cells as a Result of HIV-1III_B ENV Toxicity

- 4.7.5.1 The magnitude of this risk is uncertain, however, previous vaccine studies using HIV-1 ENV to stimulate an HIV-1 specific antibody response indicate that much larger quantities of HIV-1 ENV than are

²⁰Fearon, E., and Vogelstein, B., *Cell*, 61:759-67 (1990).

likely to be produced *in vivo* in this study have not caused any measurable side effects in treated patients.²¹

4.8 Risk Management

The risks to patients and health care professionals from the blood drawing will be minimized by careful attention to technique and close follow-up. The theoretical risks associated with gene therapy will be minimized by manufacturing practices and procedures, and quality control testing.

Adverse reactions associated with product administration will be treated symptomatically by the principal investigators. If any subjects experience adverse reactions which are incapacitating or life threatening, the appropriate interventional therapy will be administered. If recurrent grade 3 or greater BRMP toxicity is encountered, further administration of HIV-IT (V) will be suspended, and a replacement subject will be admitted into the study. If repeated grade 3 or greater toxicity is encountered, modifications in this study will be implemented as set forth in Section 4.8.1 below.

An independent monitoring board will have access to all safety data and to the product administration code. This monitoring board will evaluate adverse events to ascertain whether such events are related to treatment group or dosage and whether toxicity grade limits have been reached that would require dosage modification (see Section 4.8.1).

4.8.1 Impact of Adverse Reactions on Study Plan: Dosage Modification Schedule

- 4.8.1.1 If recurrent grade 3 or greater BRMP toxicity (see Section 4.14.0) is observed in three patients receiving the lower dose of HIV-IT (V), this study will be suspended.
- 4.8.1.2 If recurrent grade 3 or greater BRMP toxicity is observed in three subjects receiving the highest dose of HIV-IT (V), the remaining subjects will receive HIV-IT (V) at the lower dosage level (10^6 cfu).

²¹Redfield R.R. et al, *N. E. Journal of Med.*, 324:1677-1684 (1991).

4.8.2 Conditions Which May Necessitate Discontinuation of a Subject's Treatment

- 4.8.2.1 Treatment will be discontinued and a new replacement subject enrolled if a subject experiences an AIDS defining infection before follow-up Day 70 of either treatment cycle; or
- 4.8.2.2 If recurrent BRMP grade 3 or greater toxicity is encountered; or
- 4.8.2.3 If repeated CD4⁺ T-cell counts obtained at least 72 hours apart decline to an absolute level of ≤ 250 .
- 4.8.2.4 If in the attending physician's judgment the subject's condition warrants initiation of anti-retroviral drug therapy prior to follow-up Day 70 of either cycle; or
- 4.8.2.5 If there are any willful protocol violations; or
- 4.8.2.6 If a subject develops another illness which requires treatment with a drug or agent (e.g., any immunosuppressive or chemotherapeutic agent) which is likely to interfere with the activity of the gene based immunostimulatory product investigated under this protocol; or
- 4.8.2.7 If the subject elects to withdraw from the study; or
- 4.8.2.8 If, in the opinion of the attending physician, it is in the subject's best interest to withdraw from the study.
- 4.8.2.9 Anyone receiving experimental treatment will be followed for up to three years.

4.9 Termination of Study and Follow-Up Evaluation

- 4.9.1 Product administration may be terminated at any time, at the subject's request.
- 4.9.2 Subject participation may be terminated by the attending physician if justified by the subject's clinical status or if deemed in the subject's best interest.
- 4.9.3 The protocol or any individual subject's study may be terminated or modified by written communication from the Viagene Study Coordinator. The investigator will be compensated for cases completed or in progress on a pro-rated basis.
- 4.9.4 Commitment to the three-year follow-up evaluation schedule will be required of each subject who received any administration of HIV-IT (V). This follow-up data must be collected to properly evaluate safety.

4.9.5 If, in the opinion of the attending physician, subjects develop symptoms which justify further diagnostic tests and/or procedures, results of such tests and/or procedures should be reported in appropriate Case Report Forms. Such Case Report Forms will be submitted to Viagene at follow-up Day 70 of each cycle even if test results are still pending. Any follow-up test/procedure information should be forwarded to Viagene when results are available.

4.10 Potential Benefits

HIV-IT (V) may generate an immune response (CD8⁺ CTL and/or antibody) which will retard HIV-1 spread and progression towards AIDS. Information generated by this study will increase scientific knowledge about the safety and possible therapy effectiveness of a gene-transfer approach to HIV-1.

4.11 Risk/Benefit Ratio

The risk/benefit ratio seems justifiable because many precautions are taken to ensure the safety of the HIV-IT (V) product and of the placebo for recipient subjects. There is the possibility that HIV-IT (V) may stimulate an immune response that may help control HIV-1 disease progression.

4.12 Informed Consent

Subjects will receive a copy of the "Patient Brochure", fully describing the nature and purpose of the study, including the relevant background and research findings, a comprehensive description of risks, discomforts, and potential benefits. After this brochure has been reviewed by the subject and discussed with the Principal Investigator, informed consent will be obtained by the investigators prior to initiating the protocol. The Informed Consent follows in Section 5.0

4.13 Subject Study Schedule

HIV-IT Direct Vector Study Schedule

	Baseline				Treatment							Clinical Evaluation and Follow-Up									
	Day				Day							Day	Month								
	-90	-32	-18	-4	0	12	24	28	36	52	56	70	84	6	12	18	24	30	36		
History and Physical	X			X			X			X		X	X	X	X	X	X	X	X		
DTHT	X													X	X	X	X	X	X		
CTL Targets	X																				
Clinical Labs	X			X			X			X		X		X	X	X	X	X	X		
Lymphocyte Subsets	X	X	X	X		X	X		X	X		X	X	X	X	X	X	X	X		
HIV-1 Antibodies	X	X		X			X			X		X		X	X		X		X		
Viral Load	X	X		X						X		X		X	X		X		X		
CTL Assay		X		X		X			X	X		X		X	X	X	X		X		
Other Potential Surrogate Markers	X	X		X						X					X				X		
PCR/Neo				X								X			X		X				
Injections					#1			#2			#3	X			X						



5.0 CONSENT TO PARTICIPATE IN A CLINICAL RESEARCH STUDY

IMPORTANT: Every person who is invited to consent to experimental medical treatment has important rights under the Experimental Subjects' Bill of Rights (attached to this consent form) and are protected by California law (Health and Safety Code §24172). Be sure to read, understand and ask questions about these rights before consenting to participate in this study. Also, please study the patient brochure that is provided to further explain the scientific principles and other issues in this study.

STUDY

A Preliminary Study to Evaluate the Safety and Biologic Effects of an Immunotherapeutic [HIV-IT (V)] Based on Gene Transfer in Healthy Subjects Infected with HIV-1.

PROTOCOL NUMBER:	HIV-I-02-1292
PRINCIPAL INVESTIGATOR:	Jeffrey E. Galpin, M.D.
CO-INVESTIGATOR:	Dennis A. Casciato, M.D.
SPONSORED BY:	Viagene, Inc. 11075 Roselle Street San Diego, CA 92121

INTRODUCTION AND PURPOSE

I, _____, have been invited to participate in this research study ["the Study"]. The purpose of the Study is to evaluate the safety and effects on my immune system of a new investigational treatment called HIV-IT (V), HIV-ImmunoTherapeutic Vector, [the "Treatment"]. The Treatment is intended for healthy individuals infected with the human immunodeficiency virus (HIV-1).

HIV-1 infects and kills a specific group of white blood cells called "CD4 T-lymphocytes." This damage to the body's immune system leaves the infected individual susceptible to infections and cancers.

Other white blood cells, called "cytotoxic T-lymphocytes" (CTL), are believed to be important "killers" of cells infected with viruses. Thus, HIV-infected individuals could theoretically benefit from a treatment that stimulates these CTL and also produces more antibodies that are able to inactivate the virus.

HIV-IT (V) has been shown to specifically stimulate both CTL and antibody immune responses in mice and non-human primates. The Treatment has not produced toxic side effects in these animals.

The treatment involves the direct injection of a specially engineered non-disease producing virus (the "viral vector") that is disabled and contains the gene for a small portion of the HIV virus.

PROCEDURES

- A. Screening Evaluation. Only adult male or female individuals who are proven to be infected with HIV-1 and have CD4 counts greater than 400, who are free of AIDS-related symptoms, and who are healthy enough to participate in everyday activities can be selected for the study. Before I take part in the actual study, the Investigator will perform a screening evaluation to determine whether I meet all the criteria for participation. This two day evaluation includes a medical history, physical examination, urine, blood and skin tests. To conduct the skin test, a device will be pressed against the lower inside portion of my arm to insert a panel of tiny needles, just below my skin, containing standard skin test substances. Two days later my arm will be examined for temporary reactions (small bumps) to help determine whether my immune system reacts strongly enough to these substances to allow me to enroll in the study. A 100 ml blood sample (about one-half cup) will be drawn at the second visit to evaluate my general condition, my immune system and the level of HIV in my system.
- B. Exclusion. Even if I meet the qualifications for participation in the Study, I will not be allowed to take part if (1) I am unwilling to use reliable barrier birth control methods that would block HIV transmission, or (2) I am a woman who is pregnant or is breast-feeding, or (3) the Investigator determines that I have significant medical problems other than HIV-1 infection. In addition, the

Investigator may discontinue my participation in the Study at any time for any reason that the Investigator considers appropriate.

C. Medication Restrictions. While I am in the Study, I should not take any other medications without the approval of the Investigator. Specifically, I should not use any anti-retroviral drugs like AZT, ddI or ddC within 45 days prior to or during treatment or in the first two weeks of observation following the last treatment because use of these drugs would make the treatment ineffective. If I use any of these drugs thereafter, I have to inform the Investigator.

D. Study Plan.

1. If eligible for the Study, I will receive three courses of treatment consisting of injections into both arms and buttocks. The injections will be spaced at one month intervals. The injected material will be either HIV-IT (V) or a "placebo." The placebo will be an inactive substance. The "inactive substance" is the solution in which the HIV-IT (V) is normally suspended. I will be observed for two hours after each injection to identify any possible immediate side effects. If I receive a placebo, I will get a chance later to receive the gene therapy if the experimental treatment still appears to be safe at that time, and if I have not developed any AIDS defining conditions.
2. Neither I nor my physician will know whether I am receiving the active treatment or a placebo. However, this information is available if it should be necessary in an emergency.
3. I will have a medical examination and blood drawn four days before each injection day, and periodically thereafter. Additional blood and urine tests will be done 70 days, 6 Months and 12 Months after the first injection.
4. I will be asked to participate in follow-up for at least 3 years to identify long-term effects of the Treatment and to assess any effects on my immune system and medical condition. I will be examined and have blood and urine tests every six months during the second and subsequent years.

RISKS

The Treatment [HIV-IT (V)] has not been given to humans until now, so it is not known exactly which risks and discomforts might occur.

A. Potential risks or discomforts include:

1. Blood sampling may cause pain, bruising or infection.
2. The skin test may cause pain, itching, redness, blisters or sores at the application sites. Very rarely, a serious allergic reaction may occur.
3. Injections may cause pain, soreness, redness, warmth, itching, numbness or swelling at the injection site.
4. An allergic reaction to the injected materials could cause fever, chills, sweating, dizziness, shortness of breath, rash, itching, stomach or intestinal pain, nausea, vomiting, diarrhea, low blood pressure or fainting. Very rarely, some individuals could experience an allergic life-threatening reaction called "anaphylaxis"; however, this anaphylaxis is readily treatable.
5. Prior studies have been carried out at the National Institute of Health in which a similar disabled virus has been used. The disabled virus employed by Viagene has a common origin with the one used in the National Institute of Health studies; however, it is grown in dog rather than mouse producer cells and contains a different therapeutic gene, namely one aimed at instructing cells to produce an HIV-1-related protein which is intended to trigger active cytotoxic T lymphocyte responses.

B. Unforeseen complications, which are concerns that are not expected, but conceivably could occur, include:

1. Unknown disease caused by the disabled virus that is used to insert the new gene into my cells.
2. Unknown disease caused by genetic material activating an unknown virus in my body.
3. Unknown effects from undetected contaminants in the treatment.
4. Malignant diseases, such as cancer or lymphoma.
5. Changes in the genes of my sperm or ova which could have unknown effects on unborn or future children.
6. Worsening of my HIV infection.

- C. In addition, there are the other potential disappointments and uncertainties. For example, it may not be possible:
1. To measure whether my immune response has been helped by the treatment.
 2. To determine whether any immune system changes will actually help control my HIV disease.
 3. To receive the experimental treatment.

NOTE: The above complications were not observed in animal studies using HIV-IT (V) or in those related types of human gene therapy studies that have reported their results. The viral vector will be tested for safety before I am injected to reduce any risk of their causing illness or side effects. My CD4 cell count will be checked regularly and I will not be given additional injections if my CD4 count suggests that the Treatment or other factors might be making my HIV infection worse.

SAFEGUARDS

To further protect my health and safety, a Monitoring Board, independent of the sponsor and investigators, will review data about my condition after each injection to determine whether it is safe for me to receive continued injections. This Board will consist of at least three professionals, one or more of which will be a physician.

FINANCIAL CONSIDERATIONS

If I experience an adverse reaction during the Study, I should immediately contact the Investigator at (818) 344-6111. If I require medical treatment for an adverse reaction, the Sponsor, Viagene, Inc., will pay for reasonable costs of such treatment under the following conditions:

- A. The Investigator determines, based on reasonable evidence, that the adverse reaction was a direct result of the Treatment administered during the Study;
- B. The costs of treatment are not covered by any other health insurance, government health program or other third party providing coverage for health care.

Financial compensation for such things as lost wages, disability or discomfort is not available. However, I have not waived any legal rights by signing this consent form.

BENEFITS

I will neither be charged nor compensated for my participation in the Study. During the Study, the Investigator will perform numerous medical examinations and assessments. The results of these tests will generally be provided to me through my private physician upon written request.

I may receive an experimental treatment that may be beneficial to my immune system during an early stage of HIV infection when no standard treatments are available. It is possible that my condition may improve during the Study. However, this experimental Treatment may improve my immune defense system, but may not improve my condition. Even if the Treatment is effective in some patients, it may not be effective in my case.

The Treatment is an experimental substance, therefore it may not be available to me after the end of the Study. However, my participation will contribute to the search for a new treatment for HIV-1 infection.

Information from this study will help investigators learn more about immune defense mechanisms against HIV-1 infection. Results of this study may help determine the safety and possible therapeutic effect of gene therapy in humans.

ALTERNATIVE THERAPIES

No known approved treatments exist for individuals with HIV-1 infection whose CD4 counts are over 500. If my CD4 count is below 500, I may elect to receive therapy with one of the approved anti-retroviral products such as AZT or ddI; however, I must stop taking anti-retrovirals 45 days before the treatment begins and for 70 days thereafter. Thus, my participation could require my discontinuing anti-retrovirals for as long as four months. However, if a change in my condition warrants, anti-retrovirals can be initiated. Alternatively, I may choose to be treated with supportive care only.

RIGHT TO REFUSE OR WITHDRAW

My participation in this study is entirely voluntary. I may refuse to participate in the Study or I may withdraw at any time. The Investigator will provide me with new information developed during the course of the Study which may influence my desire to continue my participation. If I withdraw, I will not be penalized and I will not lose any benefits to which I am entitled. If I decide to end my participation in the Study, I should contact the Investigator who will explain the best procedure for me to withdraw.

CONFIDENTIALITY

My name will be kept as confidential as possible within the law. Research files will be maintained without identifiers (such as names) and separate from my medical record. The link will be kept by Dr. Galpin in a locked file. However, auditors from the Food and Drug Administration, and from the sponsor, Viagene, Inc. have the right to audit all records.

COMMERCIAL APPLICATION

Any commercial diagnostic or therapeutic product or process developed as a result of the Study shall be solely and exclusively the property of Viagene, Inc.

QUESTIONS AND PROBLEMS

_____ has explained this study to me and answered all my questions. If I have other questions or research-related problems, I may call Dr. Galpin at (818) 344-6111 during normal working hours. For more information regarding patient's rights in research studies, I may call The Human Subjects Office at (818) 345-2172.

If I experience a medical emergency, I should call the Investigator at (818) 344-6111 (24 hour number).

CONSENT

I have received my copy of this form to keep in plenty of time to read it and seriously consider it. I have also received the Experimental Subjects' Bill of Rights, which I have read and understand. I have had an opportunity to discuss the Study and ask the Investigator any questions I have about the Study and my participation.

Participation in this study is entirely voluntary. I have the right to refuse without any harm to my continued receipt of treatment. I also have the right to withdraw at any time.

By signing this consent form, I consent to be treated with HIV-IT (V) or a placebo under the Principal Investigator's direction.

Subject's Signature

Date

Investigator's Signature

Date

Witness' Signature

Date

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME	Galpin, Jeffrey E.	POSITION TITLE	Principal Investigator
EDUCATION (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)			
INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
University of Illinois, Chicago, IL	B.A.	1966	Cellular Biology
University of Illinois, Chicago, IL	M.D.	1970	Medicine

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

POST GRADUATE TRAINING

- 1973 - 1975 Post-doctoral Fellow, Infectious Diseases, Harbor General Hospital (UCLA), Torrance, CA
- 1975 - 1976 Fellow, National Institute of Health
- 1975 - 1977 Post-graduate Research Molecular Biology, University of California, Irvine

ACADEMIC APPOINTMENTS

- 1977 - 1980 Assistant Professor of Medicine, University of California Los Angeles (UCLA), Los Angeles, CA
- 1980 - 1981 Associate Professor of Medicine, University of California Los Angeles (UCLA), Los Angeles, CA
- 1981 - 1982 Clinical Associate Professor of Medicine, University of California Los Angeles (UCLA), Los Angeles, CA
- 1991 - Present Clinical Associate Professor of Medicine, University of Southern California (USC), Los Angeles, CA

PROFESSIONAL EXPERIENCE

- 1976 - 1981 Faculty, Infectious Diseases, Harbor General Hospital (UCLA), Torrance, CA
- 1977 - 1981 Director of Intensive Care Unit, Wadsworth V.A. Hospital (UCLA), Los Angeles, CA
- 1977 - 1981 Faculty, Infectious Diseases, Wadsworth V.A. Hospital (UCLA), Los Angeles, CA
- 1977 - 1981 Chief, Postgraduate Training in Medicine, Wadsworth V.A. Hospital (UCLA), Los Angeles, CA
- 1977 - 1981 Assistant Chief of Medicine, Wadsworth V.A. Hospital (UCLA), Los Angeles, CA
- 1981 - Present Staff Physician Medicine and Infectious Diseases, Encino Hospital, Encino, CA
- 1982 - Present Staff Physician medicine and Infectious Diseases, Sherman Oaks Hospital, Sherman Oaks, CA
- 1981 - Present Staff Physician Medicine and Infectious Diseases, Northridge Hospital, Northridge, CA
- 1981 - Present Staff Physician Medicine and Infectious Diseases, Tarzana Regional Medical Center, Tarzana, CA

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME	Galpin, Jeffrey E.	POSITION TITLE	Principal Investigator
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EDUCATION (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

AWARDS AND HONORS

- 1970 - 1971 Award Best Clinical Medicine Instructor, University of Illinois Medical Center
- 1976 R. and E. Associate National Award for Veterans Administration
- 1976 - 1978 NIH Fellowship Grant and Award
- 1979 Award National Young Administrator in Medical Program for Veterans Administration

PUBLICATIONS

1. Fischl, M., Galpin, J.E., Levine, J.D., Groopman, J.E. and Others: Recombinant Human Erythropoietin for Patients with AIDS Treated with Zidovudine. New England Journal of Medicine, 323:1069-70, October, 1990.
2. Hersh, E.M., Brewton, G., Abrams, D., Bartlett, J., Galpin, J.E., and Others: Ditiocarb Sodium (diethyldithiocarbamate) Therapy in Patients with Symptomatic HIV Infection and AIDS. A Randomized, Doubleblind, Placebo-Controlled, Multicenter Study. Journal American Medical Association, 256:1538-44, March, 1991.

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME	Casciato, Dennis A.	POSITION TITLE	Principal Investigator
EDUCATION (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)			
INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
University of California Berkley	A.B.	1960	Biophysics
University of California San Francisco	M.D.	1964	Medicine

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

POST GRADUATE TRAINING

1970 - 1971 UCLA and Wadsworth Veterans Hospital, Combined Program, Fellowship in Hematology
 1969 - 1974 Wadsworth Veterans Hospital, Los Angeles, California, Hematology/Infectious Diseases

ACADEMIC APPOINTMENTS

1974 - 1977 Assistant Chief, Medical Service, VA Wadsworth Hospital
 1974 - 1977 Chief, Postgraduate Training in Medicine, VA Wadsworth Hospital
 1976 - 1977 Acting Chief, Hematology-Oncology Section, VA Wadsworth Hospital
 1978 - 1983 Associate Chief, Hematology-Oncology Section, VA Wadsworth Hospital
 1978 - 1983 Chief, Hematology Unit, VA Wadsworth Hospital
 1973 - 1983 Associate Professor of Medicine, UCLA
 1983 - 1986 Associate Clinical Professor of Medicine, UCLA
 1986 - Present Clinical Professor of Medicine, UCLA

AWARDS AND HONORS

1960 Graduated with Honors, University of California, Berkley
 1960 Phi Beta Kappa, University of California Berkley
 1969 Citation and Commendation Medal, U.S. Army
 1972 Bringham Prize, VA Wadsworth Hospital
 1975 Commendation, Hospital Director, VA Wadsworth Hospital
 1977 Honors for Teaching, UCLA Extension, Intensive review of Internal Medicine

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME	Casciato, Dennis A.	POSITION TITLE	Principal Investigator
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EDUCATION (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

PUBLICATIONS

1. Casciato, D.A. and Lowitz, B.B. (Editors): Manual of Clinical Oncology. Second Edition, Spiral Manual Series, Boston: Little, Brown: Little, Brown & Co., Publishers, 1988 (600 pages).
2. Casciato, D.A. and Stolberg, L. PFS: A New Syndrome. Submitted to Blood, March, 1991.
3. Casciato, D.A. and Tabbarah, H.J., Chapter 72. Metastases of Unknown Origin IN C.M. Haskell (Ed.), Cancer Treatment, Third Edition, Philadelphia: W.B. Saunders, 1990, p.798-815.
4. Casciato, D.A. and Tabbarah, H.J., Chapter 73. Malignant Effusions IN C.M. Haskell (Ed.), Cancer Treatment, Third Edition, Philadelphia: W.B. Saunders, 1990, p. 815-825.

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RECOMBINANT DNA RESEARCH

PUBLICATION HISTORY

Volume	Period	Publication Date	NIH Publication Number
1	February 1975 - June 1976	August 1976	76-1138
2	June 1976 - November 1977	March 1978	78-1139
3	November 1977 - September 1978	September 1978	78-1843
3A	November 1977 - September 1978	September 1978	78-1844
4	August 1978 - December 1978	December 1978	79-1875
4A	August 1978 - December 1978	December 1978	79-1876
5	January 1980 - January 1980	March 1980	80-2130
6	January 1980 - December 1980	April 1981	81-2386
7	November 1980 - August 1982	December 1982	83-2604
8	September 1982 - September 1984	May 1986	86-2863
9	September 1984 - March 1985	May 1986	86-2864
10	March 1985 - December 1985	May 1986	86-2865
11	January 1986 - April 1987	December 1990	91-3203
12	April 1987	December 1990	91-3204
13	May 1987 - December 1988	December 1990	91-3205
14	January 1989 - July 1991	January 1994	94-3708
15	August 1991 - June 1992	January 1994	94-3709
16	July 1992 - December 1992	January 1994	94-3710
17	December 1992 - June 1993	January 1995	95-3898

NIH Environmental Impact Statement on NIH Guidelines for Research Involving Recombinant Molecules	October 1977
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